Variation in Randomly Amplified DNA Markers and Storage Root Yield in 'Jewel' Sweetpotato Clones

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Abstract. Polymorphism analysis and yield tests were conducted among 'Jewel' sweetpotato clones [*Ipomoea batatas* (L.) Lam] obtained from eight state foundation seed programs. Initially, 38 arbitrary primers generated a total of 110 scorable DNA fragments in a sample of virus-indexed plants from each clone source. The number of marker loci scored for each primer varied from one to eight with an average of 2.89. Twenty-one bands (19.1%) were scored as putative polymorphic markers based on the presence or absence of amplified products. Further estimation of variability within each clone source was accomplished by an assay of 10 sample plants per clone group by 14 marker loci generated by four selected primers. Polymorphic bands ranged from 7.1% to 35.7 % in five of eight clone groups. Field studies show variation in nearly all yield grades measured. In three tests during the 1991 and 1992 seasons, yield differences ranged from 27% to 46% within the economically important U.S. no. 1 root grade. The results suggest the usefulness of arbitrarily-primed markers in detecting intra-clonal sweetpotato DNA polymorphisms and indicate an underlying genetic cause for phenotypic variability in the crop.

Asexual propagation theoretically preserves genotypic identity and uniformity within a clonal cultivar. In the sweetpotato, the zygotic stage determines a cultivar's genotypic constitution; ensuing mitotic duplications perpetuate this unique genetic makeup. Adventitious sprouts derived from fleshy roots of sweetpotato are used for vegetative propagation, hence conservation of cultivar genetic identity is expected. However, phenotypic plasticity especially in quantitatively inherited traits is expected due to environmental effects. Several reports document the magnitude of environmental influence, expressed as genotype $\times$ environment interactions, in sweetpotato phenotypic expression (Collins et al., 1987; Huett, 1976; Kannua and Floyd, 1988; Ngeve and Bouwkamp, 1993). Nevertheless, the yield of a well-established sweetpotato cultivar can range from zero to several kilograms per plant. Slight differences in size and quality among sprouts or cuttings used in plantings cannot account for all of this observed variability (Hwang et al., 1983; Steinbauer et al., 1943). More importantly, visible qualitative variability such as root, skin, and flesh color changes are common. For instance, flesh color mutation rates in sweetpotato range between 1% and 18%, depending on the cultivar (Hernandez et al., 1964). In contrast, the estimated mutation rate in clonally-propagated potato (*Solanum tuberosum*) is one in 100,000 to 200,000 plants (Heiken, 1958). Consequently, sweetpotato foundation seed programs allocate substantial resources in maintaining the genetic uniformity of cultivars.

State foundation programs continue to maintain the genetic identity of sweetpotato cultivars using a visual selection procedure

(Dangler, 1994). Individual plants or hills of roots most phenotypically representative of a given cultivar are selected from foundation fields, and used as initial source of propagation material for the following year. Although this selection method is subjective, such an approach eliminates most visible phenotypic variants and ensures predictable performance, even though empirical evidence indicates that overall cultivar productivity may decline over time. 'Centennial,' a cultivar released in 1960 by the Louisiana Agricultural Experiment Station, has been grown annually in replicated plots since 1958 at the Sweetpotato Research Station at Chase, La., and its yield has declined by 46% over a 35-year period (unpublished data). This evidence is circumstantial due to varied plot location and changes in environment and cultural practices over time, but it illustrates the potential loss that can occur in a highly productive cultivar that is intensively selected. As expected, phenotypic selection does not detect all mutation events. This is evident by the continued appearance of off-types in foundation seed programs.

Although foundation seed providers have been in operation since the 1950s, the effectiveness of these programs in preserving the genetic constitution of specific cultivars has not been fully documented. Benchmark information is important for the following reasons: 1) assessing the efficiency of current maintenance procedures and 2) determining the need for more thorough investigation of genotypic variability within sweetpotato clones. Genotypic uniformity within a clonal cultivar depends on nucleotide sequence similarity at the molecular level. In the absence of molecular sequence data, indirect measures of determining genotypic differences that reflect underlying base pair variability are used (Nienhuis et al., 1993). These include restriction fragment length polymorphism (RFLP) analysis, variable number of tandem repeats (VNTR) fingerprinting, and polymerase chain reaction (PCR)-based techniques. In this study, we investigated the usefulness of a PCR-based polymorphism assay using arbitrary primers (Welsh and McClelland, 1990; Williams et al., 1990) in the hexaploid sweetpotato. Our objectives included the following: assess the usefulness of arbitrary primers in detecting DNA polymorphisms within the 'Jewel' sweetpotato clone, and conduct separate yield tests to determine the range of productivity in this

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cultivar. We chose 'Jewel' since several foundation seed programs have maintained this cultivar for at least 10 years following its release by the North Carolina State Univ. Agricultural Experiment Station in 1969.

Materials and Methods

Plant material. A sample of 'Jewel' fleshy roots were obtained from each of the following foundation seed programs in eight states: Alabama, California, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Virginia. The roots were acquired and bedded in 1990. Sprouts arising from these roots were cut (15-20 cm) and planted to increase the number of fleshy roots from each foundation program. This phase was also undertaken to eliminate any environmentally-induced differences among clonal stocks. Only U.S. no. 1 grade roots (5.1 to 8.9 cm in diameter and 7.6 to 22.9 cm in length) were saved for bedding the following spring (1991) and in all subsequent years. Only sprouts (transplants) of uniform length (15-20 cm) were used in field plantings.

Two sets of plant material were used in this study. The first sample set consisted of two virus-indexed, meristem-cultured plants of each clone source ($n = 16$). Plants were randomly collected from 1992 field beds, and transferred to a screenhouse for virus indexing. FAO/IBPGR guidelines were used for virus indexing (Moyer et al., 1989). This step eliminated the exopathogen *Fusarium lateritium* Nees:Fr., and any DNA-based virus or virus-like organism that might amplify and confound data analysis. The second sample set consisted of 10 clonal plants traceable to the original fleshy roots derived from each clone source ($n = 80$), and were randomly collected from 1994 field beds.

DNA extraction. Total DNA was isolated based on the method of Saghai-Marooof et al. (1984) as modified by Jarret and Austin (1994). Genomic DNA was extracted from 2 g of fresh leaf tissue from each plant. Leaf tissue was ground to a fine powder in liquid nitrogen. An isolation buffer (5 ml·g⁻¹ fresh weight) (50 mM Tris/HCl pH 8.0, 25 mM EDTA pH 8.0, 0.35 mM sorbitol, 5% (PVP-40) polyvinylpyrrolidone, 1% sodium bisulfite, and 0.2% of 2-mercaptoethanol) was added to the powder and centrifuged at 2000× g for 10 min at 4C. The supernatant and all loose debris were discarded. The pellet (crude nuclei) was resuspended in extraction buffer (5 ml·g⁻¹ fresh weight) (100 mM Tris/HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% hexadecyltrimethylammonium bromide, and 1% of 2-mercaptoethanol) followed by incubation in 60C for 30-60 min. An equal volume of 24 chloroform : 1 isoamyl alcohol (v/v) was used for extraction for 5 min with slow but constant inversion, and phases were separated by centrifugation at 5,000× g for 10 min at room temperature. The aqueous phase was removed and transferred to a new tube for DNA precipitation. About two-thirds volume of isopropanol was added, and the tube was inverted quickly several times until the DNA precipitated and then removed. DNA was dissolved in 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA, and then quantified using a UV-VIS spectrophotometer (Perkin Elmer, Norwalk, Conn.).

Arbitrary primers and amplification conditions. DNA polymorphism analysis was conducted in two phases. The initial phase involved an assay of virus-free plants in the first sample set ($n = 16$) using 40 decamer primers from kits A and F (Operon Technologies, Norwalk, Conn.). This phase was conducted in part to determine the subset of marker loci to be used for polymorphism tests in the second sample set ($n = 80$). Primers used in the second sample set were chosen based on the ability to produce at least one putative polymorphic marker locus and reproducibility of amplification. Accordingly, a sample of 14 marker loci produced by primers OPA-07

(5'GAAACGGGTG3'), OPA-09 (5'GGGTAACGCC3'), OPA-10 (5'GTGATCGCAG3'), and OPF-05 (5'CCGAATICCC3') was selected. These marker loci, scored between 10 18 and 298 base pairs (bp), represent about half of the total number of fragments produced by these arbitrary primers. DNA samples of 'Beauregard' and 'Centennial' cultivars were included in the second sample set as control fragment patterns and for outgroup comparisons.

GeneAmp PCR Reagent Kits (Perkin Elmer Applied Biosystems, Foster City, Calif.) were used in all reaction mixes. Reaction conditions were similar to those reported by Williams et al. (1990), except that we used 1.5 unit of Taq Stoffel fragment (Perkin-Elmer Applied Biosystems, Foster City, Calif.). PCR conditions consisted of 10mM Tris-HCl, pH 8.2, 50 mM KCl, 2mM MgCl₂, 0.001% gelatin, 0.1 mM of each of dATP, dCTP, dTTP, dGTP, 0.2 μM primer, and 25 ng genomic DNA per 2.5 μl reaction volume. The reaction mixture was placed in a 0.5 ml plastic reaction tube and overlaid with 25 μl of mineral oil. DNA was amplified in a thermal cycler (model 480; Perkin Elmer) programmed for 40 cycles of 1 min at 94C, 1 min at 35C, 2 min at 72C, followed by a final extension at 72C for 7 min, and then held at 4C until recovery. Amplified DNA fragments were resolved by electrophoresis (3 v/cm) for 4 h in gels composed of 1.2 % agarose (BRL, Bethesda, Md.) in 1× TAE buffer (0.04 M Tris-acetate and 0.001 M EDTA) in the presence of 0.5 μg·ml⁻¹ ethidium bromide, and photographed under UV light. The presence or absence of bands between 0.17 and 2 kb were scored from photographs. Nomenclature for marker loci represents the Operon Primer kit designation plus the estimated fragment size in base pairs (bp). A molecular weight marker (1 Kb DNA ladder, BRL) was used in visually estimating fragment size.

Analysis of amplified DNA fragments. Only fragments common in both sample sets were scored. This reduces background "noise" by possible pathogenic and viral infections of plants in the second sample set. Such introduced DNA sequences can result in amplification of artifacts (Newbury and Ford-Lloyd, 1993) that confound results and lead to erroneous interpretations. DNA extract from an infected sweetpotato sample plant can produce a unique amplified fragment not found in a pathogen-free DNA sample (unpublished data). Variability in both sample sets was expressed as percent polymorphism computed as the number of putative polymorphic markers over the total number of markers scored. In addition, fragment data in the second sample set were treated as two-state qualitative data from which genetic similarity measurements were estimated. Band data were coded as one (presence of band) and zero (absence of band) and entered into a numerical taxonomy and multivariate analysis system (NTSYS-pc), version 1.8 (Applied Biostatistics, Setauket, N.Y.). Analysis was done using similarity for qualitative data (SIMQUAL) routine to generate Dice's similarity coefficient. A triangular matrix of pairwise similarity values was generated. This matrix was subjected to a principal components analysis and a multidimensional scaling (MDSAL) algorithm from the NTSYS-pc software package.

Yield comparisons. All yield tests used a randomized complete block design with eight blocks and guard rows of 'Jewel' surrounding each planting. Twenty transplants per plot were placed 0.3 m apart within rows and 1.2m between rows in Evangeline Parish in 1991 and 1992, and at the Burden Research Plantation, Baton Rouge, La. in 1992. These plantings were harvested after 100 and 128 days in Evangeline Parish, respectively, and 134 days at Baton Rouge.

Roots were graded and weighed into U.S. no. 1, canner (2.5 to 5.1 cm in diameter and 5.1 to 17.8 cm in length), and jumbo (larger than both groups but marketable). Data were subjected to an ANOVA, and residual analysis was conducted to determine normality of data and homogeneity of variances (SAS, 1987).

Results

Amplified DNA markers. In the first sample set ($n = 16$), all primers generated fragments detected by gel electrophoresis following staining of gels with ethidium bromide. The size of the fragments ranged from 200 bp (A03) to 3,800 bp (A12). Of these, 38 primers produced a total of 110 storable markers. The number of markers scored for each primer varied from one (A-17, A-20, A-10) to eight (A-01) with an average of 2.89 bands per primer. The majority of scored bands are in the smaller fragment range, consistent with other reports concerning the use of Taq Stoffel fragment in arbitrarily-primed DNA amplification assays (Sobral and Honeycutt, 1993). This phenomenon is ascribed to the lower processivity of the Stoffel fragment compared with the native Taq enzyme (Erlich et al., 1991).

In the first sample set ($n = 16$), 21 bands (19.1%) were present in some clones but absent in others and thus were scored as putative polymorphic markers (Table 1, Fig. 1A). The presence or absence of bands was scored at markers A07-570, A07-506, and A07-451 (corresponding to about 570, 506, and 451 bp, respectively); amplified fragments at A07-344 were scored as monomorphic bands (Fig. 1A). Similarly all bands at A08-451, A08-298, and A08-259 bp were scored as monomorphic bands (Fig. 1B). Faint bands associated with both primers and other primers were not scored.

Variability within and between clone groups was detected in the second sample set ($n = 80$). Polymorphic marker loci ranged from 7.1% to 35.7% in five of eight groups of clones. Alabama and Georgia DNA samples had zero polymorphic markers whereas Mississippi and North Carolina each had 14.2% polymorphism. California, South Carolina, and Virginia had 28.6%, 35.7%, and 21.1% polymorphic loci, respectively. LA was uniquely monomorphic within the clone group but differed as a unit by 14.2%

from most samples monomorphic for all marker loci.

Using fragment data from the second sample set, a 21×21 triangular matrix of similarity values was generated using the SIMQUAL = Dice option of NTSYS-pc (data not shown). The matrix of genetic similarity measures was subjected to principal components analysis and MDSAL algorithm in NTSYS-pc. In principal component analysis, the first and second components accounted for 79.4% and 7.8% of the variance, respectively. We present the results of the MDSAL (number of dimensions in configuration space = 3) analysis using the MOD3D graphing program in the NTSYS-pc software package (Fig. 2).

Plots of putative 'Jewel' variants (shaded circles) are found in the hyperspace about the data point (solid circle) that represents most samples monomorphic for marker loci (Fig. 2). This invariant data point can be interpreted as the putative 'Jewel' DNA fragment fingerprint based on the sample marker loci. Two separate clusters are found in this hyperspace: one group includes CA11, MS3, MS7, NC6, SC6, and SC11; the other cluster includes CA3, SC2, SC5, SC10 and VA11. The LA (LA1-10) data point (solid circle) represents 10 samples that differentiated as a group from the 'Jewel' fingerprint.

Storage root yield. A summary of mean root yield by grade within each location and year is presented in Table 2. In general, the ANOVA detected significant differences among yield grades within each environment. However, no first order interactions (location $\times$ clone source, year $\times$ clone source) were observed. The highest and lowest yielders in all grades are significantly different within each year and location except canners in the 1992 Evangeline Parish plot. Variation in yield of the U.S. No. 1 grade ranged from 27% in Burden to 45% in Evangeline. Similarly, total marketable yield varied by 24% in the 1992 Evangeline plot to 34% in the 1991 Evangeline Parish experiment.

Table 1. Survey of 21 polymorphic arbitrarily-primed amplified DNA markers in eight sample 'Jewel' sweetpotato clones from the first sample set used in the study.^z

DNA marker ^y	Clone source							
	Ala.	Calif.	Ga.	La.	Miss.	N.C.	S.C.	Va.
AO3-298	+	+	+	+	+	+	-	+
AO7-506	-	-	-	+	-	-	-	-
AO7-570	+	+	+	-	-	+	+	+
AO7-451	+	+	+	-	+	+	+	+
A09-634	+	+	+	+	+	-	+	+
A10-570	-	-	-	+	+	+	+	+
A12-298	+	+	+	-	+	+	+	+
A14-396	+	+	+	+	+	+	+	-
A15-259	+	+	+	+	-	+	+	-
A15-220	+	+	+	+	-	+	+	-
A17-890	+	+	+	-	+	+	+	+
A19-698	+	-	+	-	-	-	+	-
FO1-634	+	+	+	+	+	-	-	+
FO5-506	+	+	+	-	+	+	+	+
FO6-762	-	-	-	+	+	-	+	-
FO9-201	+	+	+	+	-	+	+	-
F11-570	-	-	-	+	-	-	-	-
F14-826	+	-	-	-	-	-	-	-
F14-210	-	+	+	+	+	+	+	+
F17-670	+	+	+	+	+	-	-	+
F19-826	+	+	+	+	+	-	-	+

^y(+) Presence of amplified product, (-) absence of amplified product.

^zProvisional marker designation represents Operon primer kit designation plus the estimated fragment size in bp.

Fig. 1. Ethidium bromide stained amplification products from sample genomic DNA of sweetpotato 'Jewel' clones obtained from eight sources using primers OPA-07 (GAAACGGGTG) (a) and OPA-08 (GTGACGTAGG) (b). Lane 0 corresponds to control reaction without any template DNA. Molecular weight marker (1 Kb DNA ladder, BRL) is shown in lane M. Putative polymorphic bands (indicated by arrowheads) correspond to DNA fragments A07-506, A07-570, and A07-451. Visual comparison with molecular weight markers determines approximate DNA fragment molecular weight.

Fig 2. Three-dimensional graph of MDS analysis for genetic similarity values of the second sample set. Multiple data points (solid circle) are defined by clone source abbreviation followed by a numeral + "n" suffix, indicating number of invariant samples. Designations representing multiple data points are enclosed in parentheses. Putative marker variants (shaded circles) are identified by clone source followed by provisional numerical assignment. BEAU (Beauregard) and CEN (Centennial) (open circles) were entered as control fragment patterns and for outgroup comparison.

Discussion

Polymorphic molecular markers. We have shown the suitability of arbitrary primers in detecting DNA polymorphisms in 'Jewel' clones using a small sample size ($n = 16$) with 40 primers, and a relatively large sample size ($n = 80$) with four selected primers. Increasing marker loci in the second sample set will expand coverage of the genome and conceivably detect additional polymorphisms. Arbitrary primers have been shown to randomly sample the whole genome, including high- and middle-repetitive sequences, and single copy DNA (Williams et al., 1990). However, the dominant nature of the markers potentially limits the amount of information from polymorphism analysis using such primers, i.e., inability to distinguish between homozygous and heterozygous loci. In addition, some fragments of similar molecular weight do not necessarily contain homologous DNA segments (Smith et al., 1994). Thus a single amplification product can occasionally represent two or more loci, i.e., smaller fragments that comigrate. Furthermore, multiple genome copies inherent in the hexaploid ($2n = 6 \times = 90$) sweetpotato potentially underestimate actual variability due to the technical limitations of scoring polymorphisms based on differential band intensity on agarose gels.

For our purposes, the use of arbitrary primers in detecting intracolon polymorphisms has yielded promising results, especially in the context of earlier reports on clonal phenotypic variability. Molecular markers provide estimates of genomic variability that are independent of confounding environmental effects and should be incorporated as tools in thoroughly assessing the nature of variability in the sweetpotato. However, the magnitude of molecular polymorphisms does not necessarily reflect the degree of phenotypic variability. For instance, RFLPs that detected a 75% reduction of 25S ribosomal DNA did not phenotypically differentiate mutants vs. wild type potato plants (Landsmann and Uhrig, 1985). Nevertheless, some RFLP markers were unambiguously correlated with mutated chloroplasts in albino cereal plants regenerated from tissue culture (Day and Ellis, 1985). By efficiently screening primers in segregating plant populations, molecular markers can be linked with qualitatively and quantitatively-inherited economically important phenotypes. For example, bulk segregant analysis has been used to link RAPD markers with disease resistance in lettuce (Michelmore et al., 1991). Furthermore, quantitative trait loci (QTL) analysis with arbitrarily-primed markers is theoretically possible once RAPD-based linkage maps are established (Yu et al., 1993). For instance, arbitrary primers detected sufficient polymorphisms in a segregating population of a wild polyploid relative of sugarcane, allowing the placement of markers in linkage groups (Sobral and Honeycutt, 1993).

The MOD3D graph of the MDSCL analysis identified at least two clusters of putative 'Jewel' genetic marker variants (Fig. 2). Each of these clusters suggest similarity of either variant priming sites or altered intervening segments that are flanked by invariant priming sites. Such sequences can represent repetitive segments or conserved, but highly buffered loci in the sweetpotato hexaploid genome. On the other hand, Louisiana samples differentiated as a whole by two marker loci from the putative 'Jewel' fingerprint. These variant sequences unique to Louisiana could have been fixed by chance, a tenable situation when seed population size is small. The other data points within the hyperspace (CA10, GA9, MS9, NC2, VA4) may represent random changes within the genome detected by primers. 'Centennial' and 'Beauregard' were included in the analysis for outgroup comparisons. 'Centennial' is the paternal parent of 'Jewel' (Nugget $\times$ Centennial = Jewel). An unweighted pair-group method (UPGMA)

Table 2. Marketable yields in three locations of 'Jewel' clones obtained from eight foundation seed programs.

Clone source location, and year of trial	Yield ^a (t·ha ⁻¹)			
	U.S. no. 1	Canner	Jumbo	TMY
Evangeline (1991)				
North Carolina	11.4	6.9	0.0	18.3
Mississippi	9.9	6.2	0.0	16.2
South Carolina	8.0	6.9	0.0	14.9
Louisiana	9.6	5.1	0.0	14.7
Virginia	8.4	5.5	0.0	13.9
California	9.2	4.1	0.0	13.3
Georgia	8.2	4.9	0.0	13.1
Alabama	6.1	5.8	0.0	11.9
LSD (5%)	4.3	2.2	0.0	5.2
Evangeline (1992)				
Louisiana	25.7	8.9	5.5	40.2
California	25.3	9.2	2.3	36.8
Virginia	22.9	8.8	2.9	34.7
Alabama	22.9	9.5	1.5	33.9
North Carolina	22.6	9.5	1.6	33.8
Mississippi	22.4	7.9	2.0	32.4
Georgia	21.4	8.6	0.3	30.3
South Carolina	18.5	10.7	0.9	30.2
LSD (5%)	4.3	2.6	5.7	5.7
Burden (1992)				
Mississippi	16.2	8.3	1.6	26.2
South Carolina	15.3	8.1	0.5	24.8
Alabama	13.6	8.1	0.4	22.0
Louisiana	14.6	7.4	0.0	22.0
California	12.2	8.9	0.5	21.7
North Carolina	12.1	7.8	0.6	20.4
Virginia	11.7	6.7	0.7	19.0
Georgia	13.0	5.8	0.1	18.9
LSD (5%)	4.5	1.97	1.3	4.3

U.S. no. 1 (5.1-8.9 cm in diameter; 7.6-22.9 cm long), canner (2.5-5.1 cm diameter; 5.1-17.8 cm long), jumbo (larger vs. others, but marketable), TMY = total marketable yield.

^aMean separation in columns by LSD, $P \leq 0.05$.

with arithmetic averages clustering of the Dice similarity values (NTSYS-pc) includes the 'Centennial' and all 'Jewel' samples as expected in a separate group from 'Beauregard' (data not shown).

Genetic variability within a clone alter favorable genetic combinations and contribute to cultivar decline in the highly heterozygous sweetpotato. Reduction of productivity in clones has been generally referred to as clonal degeneration in other crops (Simmonds, 1979), more specifically as cultivar running-out in sweetpotato (Miller et al., 1959). Virus infection has also been identified as a factor in plant clonal degeneration (Richards, 1986). Specifically, Gooding (1964) cites virus as the cause of yield decline in a West Indian sweetpotato population. At the same time, Muller (1964) proposed that species without a sexual phase are not only impeded in evolution but also subject to genetic deterioration. He proposed a ratchet mechanism where mutational load can only increase compared with existing levels. This ratchet mechanism was suggested for haploid sexual organisms, but can also apply to diploid and polyploid organisms as long as these mutations are expressed in the heterozygous condition (Leslie and Vrijenhoek, 1980).

The net effect of Muller's ratchet in causing deterioration is a function of genome (more loci to mutate) and population size (Maynard-Smith, 1978). The polyploid nature ($2n = 6 \times = 90$) of the

sweetpotato can contribute to the unusually high rates of visible mutation in the crop. If a constant number of random mutations occur per generation in a haploid genome, then a triploid will experience three mutations for every two experienced by a diploid (Mogie, 1992). Thus, in the hexaploid sweetpotato, six mutation sites are theoretically possible compared to two in a diploid counterpart. At some point, the extra genome dosage may not sufficiently buffer additional deleterious mutations and also the complex interactions among mutant sites, wild type alleles, and the prevailing environment. This is synonymous to attaining a mutation/selection equilibrium in Muller's ratchet mechanism before mutational load increase. Presuming the ratchet mechanism operates, and assuming all deleterious mutations are expressed, then cultivar decline becomes a function of time.

The mode of propagation can also contribute to the crop's predisposition to genetic variability. In tropical propagation systems, the sweetpotato is virtually a perennial, where stem cuttings are collected from standing crops in a continuous planting procedure (Simmonds, 1976). In contrast, the crop is grown as an annual in the United States and in other subtropical production areas. In these areas, the sweetpotato sprout is the unit of propagation. These sprouts are derived from adventitious buds on storage roots. Adventitious buds may develop in callus, wound periderm, the vascular cambium or in anomalous cambia (Edmond and Ammerman, 1971; Fahn, 1982). Adventitious bud production, particularly from callus or anomalous cambia, originate from previously non-meristematic cells. This nonmeristematic origin can contribute to systematic variability in subsequent generations. For example, in vitro cultures established from nonmeristematic tissue in other plant species have a higher rate of variation than cultures from organized tissues (meristems) (D'Amato, 1985). Gould (1984) identified differences in the duration of various phases of cell cycles in meristematic and nonmeristematic dividing cells in tissue cultures of various plants. Such disturbances cause delay in DNA replication in heterochromatic regions and result in genetic variation (Lee and Phillips, 1988). Assuming absence of strictly regulated cell cycles, each round of sweetpotato clonal multiplication potentially introduces subtle variability that may accumulate over time. Hence, certain aspects of the sweetpotato propagation system may resemble somaclonal variation observed in in vitro systems.

Variation in yield. Differences in yield measurements suggest the range of mean productivity among clones. The absence of first order interactions for yield data suggests that the change of mean rankings across environments was not significant, and the differential response among genotypes, without rank change across environments, was also not significant. If root samples are accurate representations of each foundation program, then data can be taken to reflect the range of cultivar yield variability among clone sources (27% to 46%) across the sample environments. The differences in mean performance among clonal classes can be attributed to the following factors that act singly or jointly: 1) random and systematic fixation of deleterious and beneficial mutations, 2) interaction between the environment and mutant and wild type alleles, and 3) temporal and spatial variation in size of the source foundation seed populations.

Conclusions

At the phenotypic and genotypic level, our results suggest inherent variability still exist within clonal samples despite elimination of off-types in the source population. Many of these changes appear to be associated with the polyploid genome and compounded by the nature of the adventitiously-based sweetpotato propagation system. We are currently assessing genetic marker uniformity of seed

maintained through nodal culture (meristematic tissue origin) versus the conventional method of maintaining sweetpotato seed (adventitious origin). If propagation system plays an important role in sweetpotato clonal variability, then incorporating a method based on preexisting meristematic tissue may reduce inherent genomic variability within foundation seed programs.

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