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Novel sweet potato hybrids with enhanced anthocyanin accumulation and resistance to Sweet Potato Feathery Mottle Virus (SPFMV)

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Abstract: Sweet potato, a top five global crop with over 95% of production in developing countries, surpasses wheat, rice, and cassava in yield, nutrition, adaptability, and stress tolerance, providing more edible energy per hectare and contributing significantly to food security. Purple-fleshed sweet potatoes (PFSP) have a rich, deep purple color due to the presence of anthocyanins, which are antioxidants that have numerous health benefits. The flavor is like other sweet potatoes but can have a slightly more earthy or nutty taste, depending on the variety. Purple sweet potatoes are nutritious, being high in fiber, vitamins, and antioxidants. They can be used in various dishes, from baked and mashed forms to soups, fries, and even desserts. Molecular characterization, using SSR markers, indicated significant genetic diversity among the genotypes. Specific markers for the types of anthocyanin and resistance to Sweet potato Feathery Mottle Virus (SPFMV) were utilized to screen for promising lines. Yield trials conducted over multiple seasons showed notable differences in root count and weight, with purple-fleshed genotypes generally outperforming others, particularly during the dry season. Biochemical analysis further confirmed the high anthocyanin content in these purple-fleshed varieties, which also exhibited better yields during the dry season. Fifteen high-yielding genotypes were screened for resistance to SPFMV. The results, confirmed by SPFMV2-specific markers, showed that these genotypes were tolerant to the virus. Despite the virus's presence, these genotypes continued to perform well, making them strong candidates for cultivation in regions affected by SPFMV. Based on their morphological traits, yield performance, biochemical properties, and virus resistance, 11 purple-fleshed genotypes were identified as top performers. These genotypes hold great potential for improving sweet potato production and food security in developing countries, where they can contribute to increased yields, enhanced nutritional value, and greater resilience to viral diseases.

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1. Introduction

Sweet potato plays an important role as a major food and feed source in developing countries, accounting for over 95% of its global production (CIP, 2023). It stands among the top five crops worldwide due to its high yield, nutritional value, adaptability to diverse geographical regions, short production cycle, and resistance to various production stresses like high temperatures, water deficit, pests, and diseases (CIP, 2023). This makes it not only a significant food source but also nutritionally superior to many other staple foods. Sweet potato is cultivated in more than 100 developing countries, surpassing other root or tuber crops. It provides more edible energy per hectare per day than staple crops like wheat, rice, or cassava. Purple-fleshed varieties are rich in anthocyanins, which have antioxidant properties and potential anti-cancer and antidiabetic effects (Ayeleso *et al.,* 2016).

Purple-fleshed sweet potatoes are rich in anthocyanins with antioxidant and anti-mutagenic properties (Ayeleso *et al.*, 2016; Khoo *et al.*, 2017). These pigments are structurally stable (Suda *et al.*, 2003), with up to 22 types identified-primarily acylated cyanidins and peonidins (Terahara *et al.*, 2004; Liao *et al.*, 2019). Advanced mass spectrometry has enabled efficient characterization, facilitating large-scale screening of varieties (Tian *et al.*, 2005). Compared to orange-fleshed types, purple sweet potatoes contain significantly higher anthocyanin and phenol levels, comparable to blueberries, blackberries, cranberries, and grapes, making them a low-cost, valuable source of natural pigments.

Consumer preference for sweet potato varieties has shifted over the years from white-fleshed, high-starch types to those with purple-fleshed varieties, which are higher in protein and anthocyanin (Chandrasekara and Kumar, 2016). Purple sweet potato is an important source of dietary fiber, minerals, and vitamins. The daily dietary structure of American residents revealed that the average daily anthocyanin intake per capita was approximately 12.5 mg/day (Wu *et al.*, 2006). Therefore, purple sweet potatoes can not only be used as a green food to meet people's daily intake of cereals, but also increase the daily intake of anthocyanins to achieve health effects (ASHS, 2007).

Sweet potato virus disease (SPVD), caused by co-infection of sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV), is the most destructive viral disease of sweet potato, leading to severe stunting, curling, chlorosis, and yield losses of up to 80% (Gutiérrez *et al.*, 2003). SPFMV, the most widespread, is aphid-transmitted and often symptomless but becomes highly damaging in virus complexes (Kreuze, 2002; , Kreuze and Fuentes, 2008; Clark *et al*., 2012), while SPCSV, transmitted by whiteflies, was once mistaken for nutrient deficiency (Sim *et al.*, 2000). In the Philippines, SPVD ("kulot") is a major constraint, with incidence ranging from 10-60% (Prasanth and Hegde, 2008). Control relies mainly on resistant varieties, clean planting materials, and sanitation, as vector control is impractical and symptomless infections complicate selection. Maintaining sweet potato collections presents challenges such as genetic losses due to small plot sizes, virus infections, and significant losses during dry periods (Huaman, 1999). Cross-contamination and virus spread within clonal collections are common issues. To support the national government's food production program, efforts are directed towards assessing the genetic diversity in sweet potato germplasm collections using morphological and molecular markers and selecting varieties with high resistance to SPFMV.

The study aimed to select promising lines of sweet potato with high anthocyanin content, root yield, dry matter, and starch content, supporting commercial production and contributing to national food security. Specifically, this study aims to evaluate, select, and develop sweet potato varieties/ accessions with high anthocyanin and resistance to SPFMV suitable for commercial production.

2. Materials and Methods

Selection of purple-fleshed accession in the sweetpotato germplasm collection

A total of 27 purple sweet potato genotypes were studied. These accessions were characterized using the Revised Protocols for Sweetpotato Characterization in the Philippines, developed by NPGRL, CIP, and PhilRoots. Sixteen morphological traits related to shoots and leaves-such as leaf color and shape, as well as vine, petiole, and root morphology-were assessed in the sweet potato collection. From this collection, purple-fleshed sweet potato genotypes were selected for further evaluation in the field trial and as parents for the hybridization.

DNA isolation

Genomic DNA of the selected purple-fleshed sweet potato accessions were isolated using a modified Doyle and Doyle (1987) protocol. One gram of leaf tissue was ground with liquid nitrogen and PVP, then mixed with a CTAB extraction buffer and incubated at 65°C for an hour. The aqueous phase was treated with chloroform alcohol and centrifuged. The aqueous layer was mixed with NaCl and PEG solution, incubated at -4°C, and centrifuged again. DNA was precipitated with isopropanol, incubated overnight at -20°C, collected, washed with ethanol, and air-dried. The DNA pellet was resuspended in TE buffer with RNAse A and incubated at 37°C. DNA quality was assessed using a Biotek Epoch™ UV-VIS spectrophotometer and agarose gel electrophoresis and visualized under UV light with a Clinx GenoSens 1510 system.

Primer selection and polymerase chain reaction

Gene-specific molecular markers for anthocyanin biosynthesis (Table 1) were selected from published studies (Mano *et al.*, 2007; Park *et al.*, 2015; Khan *et al.*, 2016; Choudhury *et al.*, 2019) and synthesized for screening sweetpotato genotypes. Anthocyanin-related markers were amplified in purple-fleshed varieties, while β-carotene markers were amplified in orange-fleshed ones. DNA was normalized to 60 ng/µl, and PCR conditions were optimized based on reported protocols with annealing temperatures adjusted accordingly. Amplification products were resolved using 6% polyacrylamide gel electrophoresis (PAGE) stained with GelRed™ and visualized under UV light with the GenoSens 1510 system.

RNA Isolation for resistance to SPFMV screening

Symptomatic leaf tissues (100 g) were homogenized in a frozen mortar and pestle, and total RNA was extracted using Trizol® reagent following the manufacturer's protocol. After phase separation with chloroform and precipitation with isopropanol, RNA pellets were washed with ethanol, air-dried, and resuspended in RNase-free water. First-strand cDNA was synthesized using the SuperScript™ III First-Strand Synthesis System with oligo(dT) primers, following standard conditions of denaturation, reverse transcription at 50°C, and termination at 85°C. The resulting cDNA was stored at -21°C and

Table 1 - List of gene-specific markers used for anthocyanin content screening across sweet potato (*Ipomoea batatas*) genotypes

Primer	Gene name	Gene function	Forward sequence	Reverse sequence	Ta (°C) literature	Ta (°C) Optimized	Product length (bp)	Literature cited
IbMYB1-FA/RA2	MYB proto-oncogene, transcription factor	codes for transcription regulator in anthocyanin synthesis	TATGGTCGGGATCGTCTTCG	TTCTGAAGATGGGTGTTCCAT	56	56	800	Mano *et al.*, 2007
CHS-F/R	chalcone synthase	flavonoid/isoflavonoid biosynthesis pathway	GGACTACCAGCTCACCAAGC	GTCCTCCACTTGGTCCAGAA	56	56	400-800	Mano *et al.*, 2007
CHI-F/R	chalcone isomerase	isomerization of naringenin chalcone into its corresponding (2*S*)-flavanones	GTTAAGTGGAACGGGAAAAG	GAGACGACCGTTTGTGGAAT	56	56	800	Mano *et al.*, 2007
F3H-F/R	flavanone-3-hydroxylase	flavonoid biosynthetic pathway	CGAGATTCCGGTGATATCGT	GGGGCATTTTGGGTAGAAAT-	56	56	900	Mano *et al.*, 2007
DFR-F/R	Dihydroflavonol 4-reductase	flavonoid biosynthetic pathway	TCCTGGGAACACAAAGAAGG	GAGCTTCGCAGAGATCATCC	56	56	1300	Mano *et al.*, 2007
ANS-F/R	anthocyanidin synthase	catalyzes the penultimate step in the biosynthesis of the anthocyanin	ATTTTCGCGGAGGAAAAGAT-	CTTCCTTCTCCAGCCTTCCT	56	56	800	Mano *et al.*, 2007
3GT-F/R	flavonoid 3-glucosyl-transferase	flavonoid biosynthesis	AAGTATCGATCGGCGAAATG	CACGATATGGCCTCCAGAGT	55	55	800	Mano *et al.*, 2007
VP24-F/R	encoding vacoular protein	Precursor in Anthocyanin-Producing Cells	CTTGACACTGCCCTCCAGTATG	ACGAGCAAGCTCCAACATAACA	56	56	800	Mano *et al.*, 2007
IT 4	sporamin	endopeptidase inhibitor activity	CCATACCAGCTCGGATTTGT3	TGGATGCCAACCTTAACTCC3	55	55	234	Choudhury *et al.*, 2019
IT 666	R2R3 type MYB gene	flavonoid 3'-hydroxylase of Ipomoea tricolor	GCGAATTTAGTCCCGATGAA	CGGTGTTTTCCGTGATTTCT3	52	52	479	Choudhury *et al.*, 2019

subsequently used for screening sweet potato genotypes for SPFMV resistance (Table 2).

Field evaluation of purple-fleshed sweet potato accessions

The twenty-seven purple-fleshed sweet potato genotypes were evaluated in preliminary yield trials using a randomized complete block design (RCBD) with three replicates. The first trial was conducted during the dry season (November 2020-March 2021) and harvested 105 days after planting. Storage roots were classified as marketable or nonmarketable, and root number and weight were recorded per replicate. Morphological traits (skin and flesh color) were assessed, while dry matter, starch, and anthocyanin contents were analyzed at the Analytical Service Laboratory, IPB, CAFS, UPLB. A second field evaluation was conducted during the wet season (May-September 2021) using the same design and procedures.

3. Results and Discussions

Morphological characterization of sweet potato genotypes

Morphological characterization for foliage and vine was conducted using sixteen descriptors based on the revised protocol for sweet potato characterization in the Philippines. Traits such as leaf color and shape, vine and petiole color pigmentation, and number and type of lobes were noted and scored. Some of the predominant phenotypic traits observed from leaves across genotypes were the triangular leaf outline and semi-elliptical central lobes. Leaf color variations were mostly green or purple, with veins that can also be green or strongly pigmented with anthocyanin. There are also genotypes with both green mature leaves and purple young leaves. Some genotypes even display a variation of leaf shape and lobe number on the same plant (Fig. 1).

Table 2 - List of gene-specific markers for screening of SPFMV-resistant sweet potato (*Ipomoea batatas*) genotypes

Primer	Forward sequence	Reverse sequence	Ta (°C)	Product length (bp)	Literature cited
SPFMV	CACTTCAGTGACGTTGCTGA	GCACACCCCTCATTCCTAAG	60	319	Sivparsad and Gubba, 2013
SPFMV	TGGGGTTATGATGAACTTCTTC	TTCTGGAATGRYTGCGGGTTG	60	400	Zhao *et al.*, 2020
NIB1536+	TAATGAAATGTAYGATGATAG	TTAAAGGCATACTAAAGATAA	60	1051	NCPN, 2016
SPFMV	TCTAATGAGAACACTGAATT	TTGCACACCCCTCATTCCTAAG	60	1051	Jiang *et al.*, 2018
CP1S	AGTGGGAAGGCACCATACATAGC	GCAGAGGATGTCCTATTGCACACC	-	960	Prasanth and Hegde, 2008
CP2S	TCTAGTGAACGTACTGAATTCAAAGA	ATTGCACACCCCTGATTCCTAAGA	-	960	Prasanth and Hegde, 2008

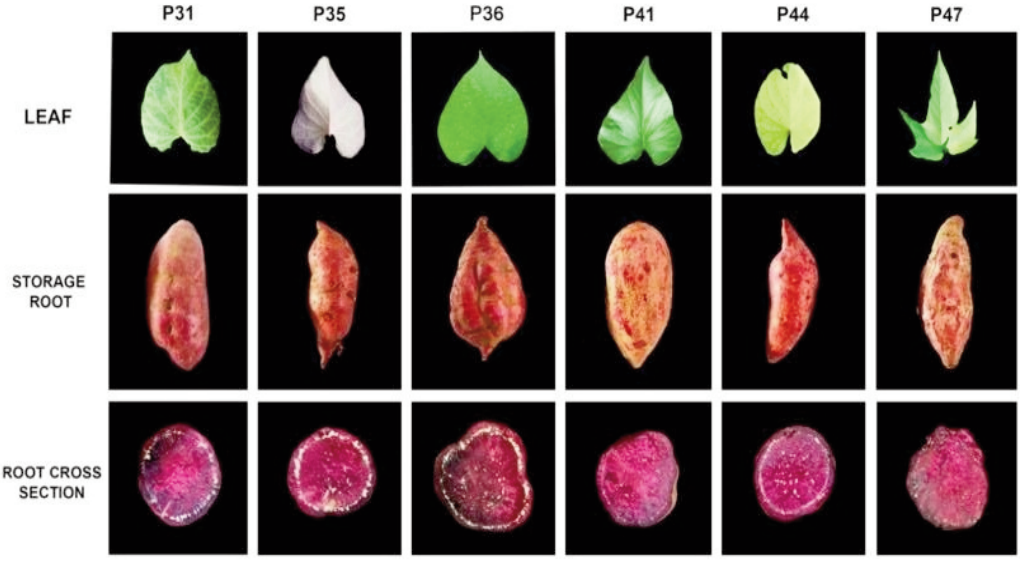

Fig. 1 - Morphology of top-performing purple-fleshed sweet potato genotypes subjected to general yield trial.

On the other hand, root morphological assessment was carried out after harvesting. The presence of purple pigmentation in the root flesh denotes high anthocyanin biosynthesis. Hence, flesh color was scored through their cross-sections. Most of the purple-fleshed genotypes were characterized as intermediate purple to strongly pigmented with anthocyanin, and a few individuals were recorded with pale purple color. Interestingly, similar to the check variety, genotypes that showed high pigmentation of purple color during the dry season were observed to have lighter flesh color or less pigmentation during the wet season. This might be associated with some environmental factors, such as excess amount of moisture during wet season considering that color intensity depends on environmental conditions (Huaman, 1999). For accurate quantification of dry matter, starch, and anthocyanin of the storage roots of each genotype per replicate were sent to Analytical Service Laboratory of Institute of Plant Breeding. Moreover, storage root shape, size, and skin color were also noted. Most genotypes have red purple to dark purple skin while the shape varies from elliptic, round elliptic, ovate, obovate, and oblong. Most genotypes showed normal root surface, but some showed some defects such as horizontal constriction (P25), longitudinal grooves (P36), and vein-like skin (P47). These defects occur normally as the result of environmental factors such as soil type or presence of excessive amount of water (Huaman, 1999).

Molecular characterization of sweet potato genotypes

Genomic DNA was isolated using the modified Doyle and Doyle (1987) protocol. Intact bands were generated using 1% AGE gel, indicating absence of degradation (Fig. 2). Moreover, quality and quantity assessment through spectrophotometry showed an A_{260}/A_{280} ratio reading of 1.8-2.0 indicating good quality DNA. Normalization of template concentration was done by dilution of DNA to a final concentration of 60ng for all samples. Moreover, primer selection and synthesis were done to carry out the molecular characterization of sweet potato genotypes. Fifty-four SSR markers were selected from the study of Meng *et al.* (2018), Amoanimaa-Dede *et al.* (2020), and Naidoo *et al.* (2022).

Fig. 2 - Genomic DNA of sweet potato representative genotypes resolved in 1% agarose gel.

DNA-based markers are crucial in expediting the timeline of any breeding program. Therefore, in the initial screening of sweet potato varieties for high anthocyanin, gene-specific markers were selected from the study of Mano *et al.* (2007), Park *et al.*, (2015), Khan *et al.* (2016) and Choudhury *et al.* (2019). Amplification of these genes was carried out across the advanced lines. Anthocyanin-related markers were amplified across purple-fleshed genotypes. Presence and absence of bands were recorded for each genotype. All genotypes generated positive amplification for each marker, with a few having one or two missing bands. This indicates the presence of anthocyanin biosynthesis across genotypes (Table 3). In addition, screening for SPFMV resistance was conducted after selecting the top-

Table 3 - Presence and absence of bands from purple-fleshed genotypes using anthocyanin biosynthesis gene specific primers

Accession Number	F3H	CHS	ANS	IBM	IT4
P25	+	+	+	+	+
P27	+	+	+	+	+
P28	+	+	+	+	+
P29	+	+	+	+	+
P31	+	+	+	+	+
P32	+	+	+	+	+
P33	+	+	+	+	+
P34	-	+	+	+	+
P35	+	+	+	+	+
P36	+	+	+	+	+
P37	+	+	+	+	+
P39	+	+	+	+	+
P40	+	+	+	+	+
P41	+	+	+	+	+
P42	+	+	+	+	+
P43	+	+	+	+	+
P44	+	+	+	+	+
P45	+	+	+	+	+
P46	+	+	+	+	+
P47	+	-	+	+	+
P48	+	+	+	+	+
P49	+	+	+	+	+
P50	+	+	+	+	+
P51	+	+	+	+	+

performing genotypes in the yield trial (Fig. 3). Gene-specific markers for SPFMV were selected from the study of Prasanth and Hegde (2008), Sivparsad and Gubba (2013), Jiang *et al.* (2018), and Zhao *et al.* (2020), and were subjected for synthesis.

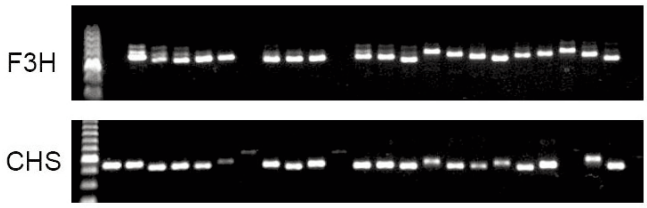

Fig. 3 - Representative gel of gene-specific markers for anthocyanin (F3H, CHS).

Yield trial of sweet potato genotypes

Yield for each individual genotype was counted and recorded for all replicates after harvesting. Analysis of variance was done for each recorded character (Table 4). Significant difference was observed from all parameters.

Significant increase in yield was observed during the dry season for both tuber weight and count (Fig. 4). Eight purple genotypes generated significantly higher values than the check variety for root storage count, with a range of 45.33 to 76.0 for dry season and 25.42 to 42.10 for tubers during the wet season. Eleven purple genotypes were accounted to have higher root weight values with 3.93 kg - 16.37 kg per 6 m plot. This is higher than the check variety with approximately 3.83 kg yield for dry season. Significant decrease was observed during the wet season, where 93% of the purple genotypes obtained lower values than the check variety. Highest yield performance was obtained from haponitaxhaponita[2] (P34) and HAPONITA X NSIC SP20 (P36) for dry and wet season, respectively.

Results showed that yield of purple genotypes were significantly higher during the dry season than

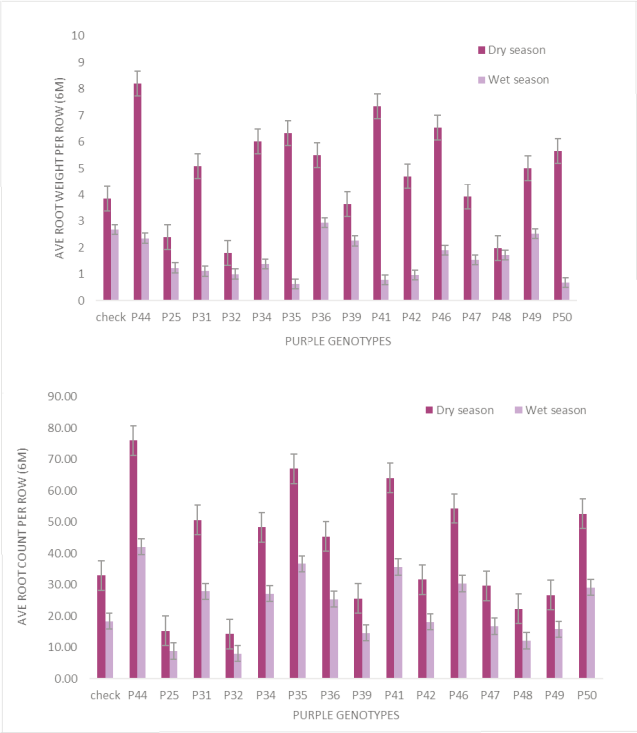

Fig. 4 - Relative root weight (top) and count (bottom) of purple genotypes harvested during dry and wet season

the wet season. However, it is important to note that sweet potato is of tropical origin and thrives better in dry season. This is why yield evaluation for wet season is a selection of at least reasonable quantity in contrast to dry season where excellent yield performance can be obtained and selected across genotypes (Wilson *et al.,* 1989). This proves that root formation of sweet potato is highly dependent on optimum environmental conditions. It was also evident in the morphology of several roots from wet season where defects in skin surfaces such as horizontal constriction, longitudinal groove, and vein-like skin were detected. This might be associated with factors like the relatively high moisture content

Table 4 - Analysis of variance for yield based on root count and weight per planting season

Purple genotype	Sum Sq	Mean Sq	F value	Pr(>F)
Root count dry season	16044.8	1069.65	5.1686	5.633e-05 ***
Root count wet season	2760.65	184.043	2.854	0.006617 **
Root weight dry season	164.220	10.9480	3.6479	0.001244**
Root weight wet season	25.5321	1.70214	3.094	0.003823 **

***, **, *, · means different significance for p≤0.001, 0.01, 0.05, 0.1, respectively.

present during wet season or even temperature differences.

Yield trial was conducted for the wet season across purple-fleshed sweet potato genotypes. Genotypes were significantly different for root weight (α=0.05), which ranges from 3.033 to 0.1 kg. Tukey's Honest Significant Differences (HSD) were carried out across accessions that grouped 16 purple individuals with SP34 and Inube check varieties. Among top performing genotypes, Nick732, P35, P44, P39, P47, and P41 obtained higher values than both purple check varieties (Fig. 5).

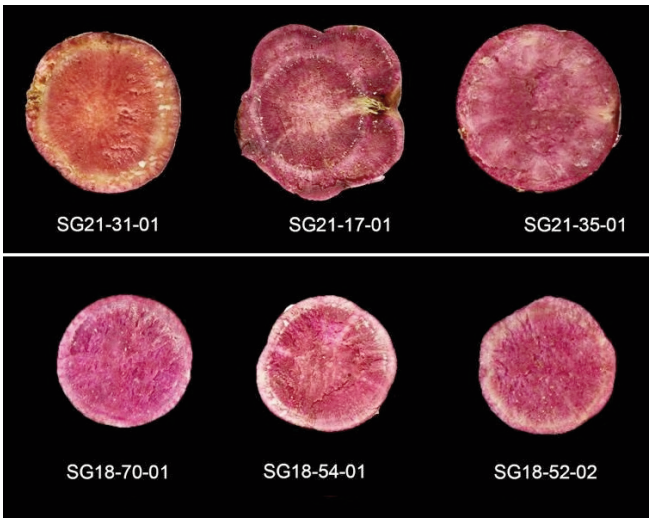

Fig. 5 - Root cross sections of selected, purple-fleshed sweet potato genotypes after field evaluation.

Evaluation of sweet potato genotypes for anthocyanin, dry matter and starch content

Results showed significant differences for all parameters except starch content for dry season (Table 5). Anthocyanin content of purple genotypes for dry season ranged from 12.35 mg L^{-1} to 138.044 mg L^{-1} with the highest value obtained (Table 6).

Table 6 - Biochemical profile of representative top performing purple-fleshed sweet potato lines for wet and dry season

CODE	Anthocyanin	%Dry matter	%Starch
Dry season			
P35	138.0441 a	25.4062	82.31325
P39	117.8663 ab	26.01484	79.35187
P41	91.45423 abc	29.23401	82.25589
P36	68.82724 bcd	29.60067	81.38915
P47	67.96446 bcde	31.05805	78.60501
P32	50.29147 cde	31.35445	80.13882
P44	48.8999 cde	33.96263	81.12375
P46	47.4805 cde	30.75377	80.54859
CHCK	42.24818 cde	33.42631	79.59258
P34	41.10709 cde	32.46437	82.35191
P48	33.89874 de	29.38918	81.73909
P31	33.45343 de	36.33059	81.9476
P42	28.9169 de	27.35473	81.76478
P50	22.79398 de	32.25506	82.12245
P25	16.36491 de	34.43242	80.99422
P49	12.35717 e	30.25301	81.86431
Wet season			
P35	65.29264 a	29.70628	63.52073
P34	39.40937 b	32.00372	68.95282
P44	36.8767 bc	34.96165	79.35083
P39	35.12332 bcde	30.64573	71.73507
P47	33.03596 bcdef	34.2485	78.36539
P41	31.36607 bcdef	31.46032	79.80127
P31	22.09819 bcdef	38.14614	77.95936
CHCK	17.45033 bcdef	34.02202	73.97153
P32	15.27948 bcdef	36.1835	73.27699
P36	15.22382 bcdef	26.48254	69.87282
P42	14.33321 cdef	32.28941	74.80796
P25	11.60572 def	37.52476	78.94269
P50	11.23 def	56.79378	49.16729
P49	96.01859 ef	31.08886	73.01925
P46	92.4005 ef	34.21978	78.75343
P48	74.86667 f	31.64359	78.61174

Means with the same letter are not significantly different.

Table 5 - Analysis of variance for yield based on root count and weight per planting season

Purple genotype	Sum Sq	Mean Sq	F value	Pr(>F)
Dry season				
Anthocyanin	55161	3677.4	11.3160	3.529e-08 ***
Dry matter	394.57	26.3047	8.9181	4.695e-07 ***
Starch	54.747	3.6498	0.7608	0.70612
Wet season				
Anthocyanin	10858.6	723.91	11.2722	2.494e-08 ***
Dry matter	409.15	27.277	2.3945	0.02128 *
Starch	952.60	63.507	2.0123	0.051803 ·

***, **, *, · means different significance for p≤0.001, 0.01, 0.05, 0.1, respectively.

Percent dry matter across hybrids ranges from 20.96 to 36.33 while percent starch content ranges from 76.23 to 82.35. Relatively lower anthocyanin content was obtained during the wet season. Anthocyanin content ranges from 9.60 to 65.29 mg L^{-1} for purple genotypes. Five accessions of purple genotypes (P35, P44, P39, P47, and P41) were consistently higher than the check varieties across two seasons. Results were consistent with the morphological scoring for flesh color, in which higher color pigmentation was observed during the dry season relative to results obtained during the wet season.

Selection of promising sweet potato lines

Accessions and hybrids with high yield, high dry matter, starch content and high anthocyanin levels were selected for an advanced yield trial which was carried out mainly on the basis of storage root weight and anthocyanin content across genotypes. Significant difference groupings (Tukey's test) based on root weight shown in Table 7 revealed the top yielding genotypes. Individuals with higher yield and/or in the same group as the check variety were considered. The advanced yield trial was then carried out in randomized complete block design (RCBD) in four replicates, each of which consists of two 6-meters plots. Each plot consists of 21 cuttings with 0.3-meter distance between hills and 1 meter distance between rows. Two check varieties namely SG08-09-11 and JK09-11-08 for purple genotypes were included in the trial. Figure 6 showed the morphology of the storage root and root cross section of representative genotypes that were selected for GYT.

Significant differences for root yield were observed across purple genotypes in dry and wet seasons trials (Table 8). Seven purple-fleshed genotypes obtained consistently higher root yield values as compared to check varieties across seasons.

Table 7 - Tukey's Honest significant difference groupings of top-performing purple genotypes based on root weight

Purple genotype code	Weight (kg)
Dry season	
P15	8.20 a
P41	7.33 ab
P34	6.91 abc
P46	6.53 abc
P35	6.33 abc
P50	5.67 abc
P36	5.50 abc
P31	5.10 abc
P49	5.00 abc
P42	4.70 abc
P47	3.93 abc
check	3.83 abc
P39	3.63 abc
P25	2.40 bc
P48	1.97 c
P32	1.80 c
Wet season	
P36	2.93 a
check	2.68 ab
P49	2.52 ab
P15	2.35 ab
P39	2.26 ab
P46	1.90 ab
P48	1.72 ab
P47	1.53 ab
P34	1.38 ab
P25	1.23 ab
P31	1.12 ab
P32	1.00 ab
P42	0.97 ab
P41	0.78 ab
P50	0.68 ab
P35	0.63 b

Means with the same letter are not significantly different.

Table 8 - Analysis of variance (ANOVA) on root weight across selected, purple-fleshed genotypes

	Sum Sq	Mean Sq	F value	Pr(>F)
Dry season				
Purple genotype	339.52	30.865	7.419	3.723e-05 ***
Rep	10.93	5.466	1.314	0.289
Wet season				
Purple genotype	49.385	44.895	6.309	0.000418 ***
Rep	8.698	43.492	61.116	0.010003 ***

***, **, *, · means different significance for p≤0.001, 0.01, 0.05, 0.1, respectively.

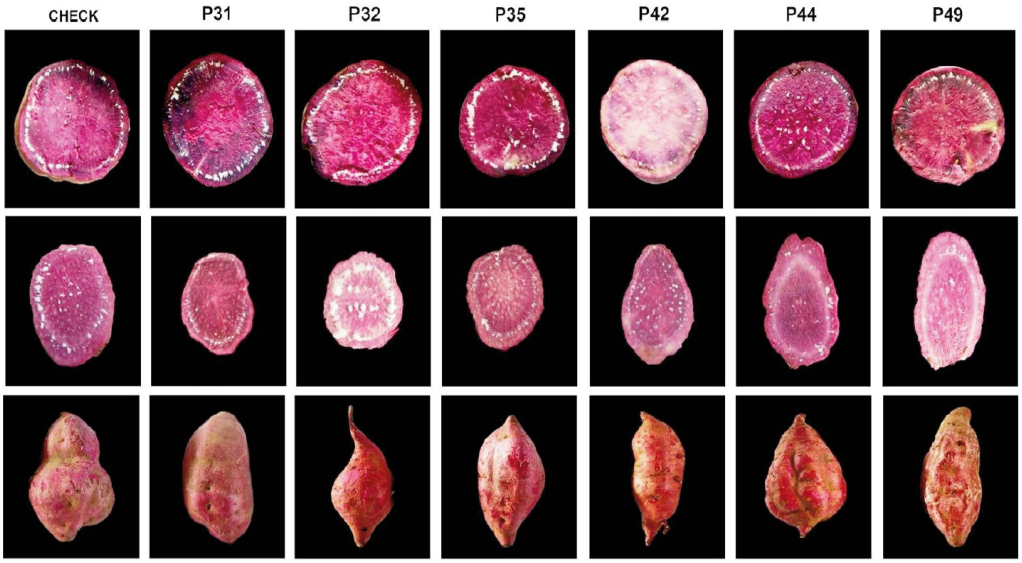

Fig. 6 - Root cross section of selected purple-fleshed sweet potato hybrids planted in the dry (upper) and wet (middle) season of 2022; bottom whole storage root.

With the consideration of their biochemical profile, a total of 11 purple-fleshed hybrids were considered high performing. Yield performance, morphological, biochemical analysis, and virus screening using PCR of the selected purple fleshed genotypes are summarized in Table 9.

Table 9 - Summary of general yield trial results and Tukey's Honest significant difference groupings of representative purple and orange genotypes based on root weight

Purple genotype	Ave. Yield (t/ha) dry season	Ave. Yield (t/ha) wet season
P31	1.9136 d	0.463 c
P34	4.6605 c	1.4815 b
P35	1.1728 d	0.2778 c
P36	12.2839 a	4.074 a
P41	3.2716 c	0.0972 c
P44	6.4814 bc	2.0988 ab
P46	5.5864 bc	1.2654 b
P47	3.5185 c	0.3241 c
P49	4.2592 c	0.2469 c
P50	6.2592 bc	0.2778 c
P15	5.9105 bc	0.3395 c
check1	8.1635 b	0.3395 c

Means with the same letter are not significantly different.

Screening for SPFMV resistance

Fifteen (15) high-performing, purple-fleshed sweet potato genotypes were selected and screened for resistance to Sweet Potato Feathery Mottle Virus (SPFMV) using specific primers. Initially, a field survey was conducted to identify vines exhibiting symptoms consistent with SPFMV infection, such as leaf curling and vein clearing. Samples from symptomatic plants were collected for further analysis. Detection of SPFMV was carried out using Reverse Transcription Polymerase Chain Reaction (RT-PCR). Plants testing positive for SPFMV were potted and maintained under greenhouse conditions to serve as positive controls. Concurrently, cuttings from the selected genotypes were propagated in plastic pots and placed in a screen cage for resistance screening.

SPFMV was isolated and inoculated into test plants via insect transmission using aphids (*Aphis gossypii*). The plants were incubated in sealed screen cages for two weeks to allow viral infection. After incubation, total RNA was extracted from infected plants using Trizol©, which yielded high-quality RNA isolates. Reverse transcription PCR was then performed to confirm the presence of the virus in each genotype. Complementary DNA (cDNA) synthesis was carried out following the protocol for the SuperScript™ III First-Strand Synthesis System (Invitrogen™, USA). Amplification of the cDNA was achieved using gene-specific primers. Among these, the SPFMV2 primers developed by Sivparsad and Gubba (2013), which target coat protein genes, produced positive amplification results.

All 15 genotypes evaluated in this study were identified as high-yielding and top-performing under

experimental conditions (Fig. 7). The detection of SPFMV in certain genotypes highlighted their tolerance to the virus, as these plants maintained strong performance despite infection (Fig. 7). Conversely, genotypes that tested negative for SPFMV were considered putatively resistant, as they showed no signs of infection during the testing process. Of the 15 genotypes, the presence of the viral gene was confirmed in several through the appearance of characteristic bands during PCR amplification.

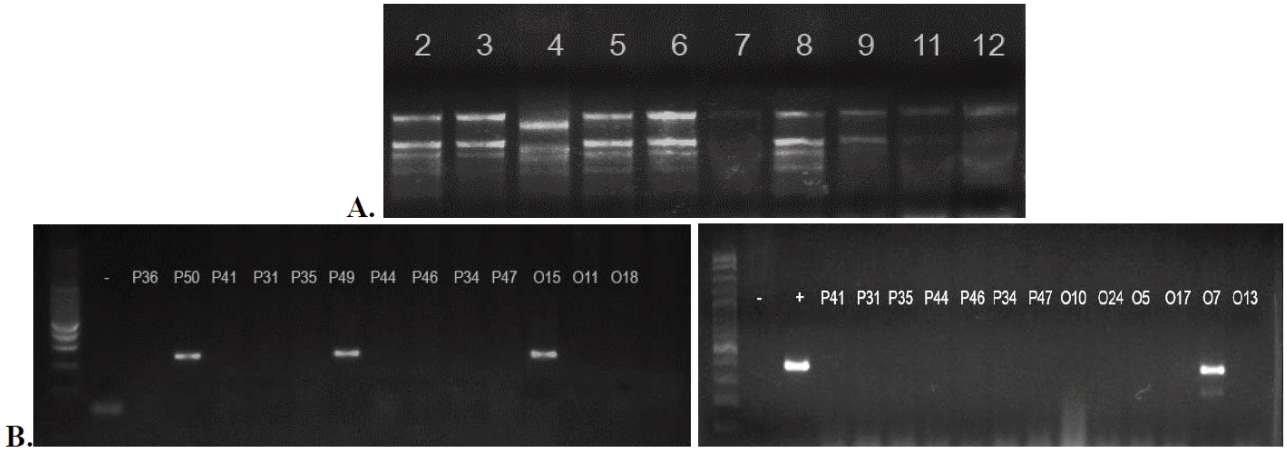

Fig. 7 - Representative total RNA (A) of sweet potato genotypes. Screening of purple-fleshed sweet potato genotypes for SPFMV (B) resistance using identified primers for the viral coat protein.

These findings underscore the importance of integrating both tolerance and resistance traits into sweet potato breeding programs. By doing so, the resilience and productivity of sweet potato crops can be significantly enhanced, ensuring better performance even under conditions of viral pressure or intensity and frequency of exposure of a plant to viruses and their vectors.

4. Conclusions

Sweet potato is recognized as one of the five leading crops globally in terms of production. The crop is increasingly popular, producing more edible energy per hectare per day than wheat, rice, or cassava. Despite its benefits, maintaining sweet potato collections in the field presents challenges. The maintenance of field genebanks is costly, and crops are exposed to diseases, pests, and environmental stresses. The Sweet Potato Virus Disease Complex (SPVD), caused by SPFMV and SPCS, is particularly devastating, reducing yields by up to 80%. This study aimed to select promising purple-fleshed sweet potato genotypes that are high yielding, with high dry matter, starch and anthocyanin contents and has resistance to SPFMV. The presence of purple pigmentation indicates high anthocyanin content. Gene-specific markers for anthocyanin, and SPFMV resistance were used to screen the sweet potato lines. Molecular characterization using these gene specific markers for anthocyanin showed high polymorphism, indicating significant genetic variation. Yield trials showed significant differences in root count and weight across genotypes. Purple and orange genotypes performed better in the dry season. Biochemical analysis revealed high anthocyanin and beta-carotene content, with dry season yields being higher. Fifteen high-yielding genotypes were screened for SPFMV resistance. Positive amplification of SPFMV2 markers indicated tolerance to the virus, allowing these genotypes to perform well even in the presence of the virus. Based on their morphological traits, yield, biochemical profiles, and SPFMV response, 11 top-performing, purple-fleshed genotypes were selected for future breeding programs (Table 10). These genotypes hold promise for enhancing sweetpotato production and supporting food security efforts.

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Table 10 - Summary of morphological characteristics, yield, biochemical profile, gene marker profile, and SPFMV response of the selected, purple-fleshed top performing genotypes

Accession purple genotypes	Morphological characteristics					Dry seaon				Gene markers for anthocyanins					
	Leaf lobing	Foliage color	Skin color	Flesh color	Maturity days	GYT (kg)	Anthocyanin	Dry matter	Starch	F3H	CHS	ANS	IBM	IT4	SPFMV reaction
P44	Semi-elliptic	Green leaf and vine	Purple red	Purple	105-120	6.481	48.900	33.963	81.124	+	+	+	+	+	-
P50	Semi-elliptic	Green leaf and vine	Purple red	Purple	105-120	6.259	22.794	32.255	82.122	+	+	+	+	+	+
P49	Semi-elliptic	Purple vine, green leaf	Purple red	Purple	105-120	4.259	12.357	30.253	81.864	+	+	+	+	+	+
P46	Semi-elliptic	Green leaf and vine	Purple red	Purple	105-120	5.586	47.480	30.754	80.549	+	+	+	+	+	-
P36	Semi-elliptic	Purple vine, green leaf	Purple red	Purple	105-120	12.284	68.827	29.601	81.389	+	+	+	+	+	-
P34	Semi-elliptic	Green leaf and vine	Purple red	Purple	105-120	4.660	41.107	32.464	82.352	+	+	+	+	+	-
check1	Triangular	Green	Purple red	Intermediate purple	105-120	8.164	42.248	33.426	79.593						
P5	Semi-elliptic	Green	Purple red	Intermediate purple	105-120	0.97	48.260	38.930	66.870	+	+	+	+	+	-
P16	Semi-elliptic	Yellow	Pink	Pale purple	105-120	2.00	23.787	25.563	59.063	+	+	+	+	+	-
P4	Toothed	Greyish green	Purple red	Intermediate purple	105-120	0.60	20.057	40.045	62.695	+	+	+	+	+	-
M4	Triangular	Green	Purple red	Intermediate purple	105-120	0.93	23.907	30.341	81.799	+	+	+	+	+	-
P9	Semi-elliptic	Green	Purple red	Intermediate purple	105-120	1.20	89.599	29.520	59.510	+	+	+	+	+	-
Check (INUBE)	Triangular	Green	Purple red	Dark purple	105-120	0.43	98.690	39.110	69.215						

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