

Morphological characterization and SSR-based genetic diversity of *Axonopus compressus* mutants

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Abstract

Axonopus compressus mutants require characterization for germplasm identification and parent selection. This study combined morphological evaluation and SSR marker analysis in characterizing phenotypic variation and genetic diversity, aiming to distinguish seven *A. compressus* variants. The plant materials included the original species (A-0) and six mutant lines (A-1, A-40, A-46, A-91, A-122, A-D). Fifteen morphological traits were measured, showing big phenotypic variation among variants (CV: 1.62% to 59.73%). SSR-based UPGMA clustering separated the variants into three genetic clusters (Jaccard distance: 0.200 to 0.857; PIC: 0.245 to 0.571), providing complementary evidence to the morphological differentiation among variants. Morphological and SSR analyses improved germplasm discrimination and breeding grouping: A-D for compact dwarf growth with strong rooting; A-40, A-122, and A-46 for rapid ground cover and high turf density; and A-1 and A-91 for high biomass production.

1. Introduction

In tropical and subtropical regions, breeding and management emphasize rapid ground cover, good recuperative ability, and low-input performance [1]. *Axonopus compressus* is widely used because of its good shade tolerance and low maintenance requirement [2]. Germplasm characterization is essential for reliable identification and parent selection, thereby supporting breeding and cultivar registration of *A. compressus* in tropical environments.

The suitability of turfgrass can be assessed through phenotypic performance and genetic information [3]. Morphological evaluation is one of the most basic elements for germplasm resource characterization, inexpensive, intuitive and intimately related to agronomic performance [4]. However, morphology traits may be influenced by environment and management which may lead to inaccurate genetic relatedness [5]. As supplementary, molecular markers such as simple sequence repeat (SSR) markers can provide information about genetic relationships and diversity that could not be revealed from phenotypic analyses. Morphological and molecular mark methods can improve germplasm discrimination in turfgrasses [6, 7].

Mutation breeding has been continuously adopted to improve turfgrass performance, such as useful growth habit, and enhanced stress tolerance [8–10]. Mutagenesis can amplify genetic variation, but systematic identification is required to screen out stable and useful mutant lines [11]. In mutation breeding, mutant selection depends on quantitative assessment of divergence using complementary phenotypic evaluation and molecular markers [12, 13]. However, in *A. compressus*, SSR-based surveys have mainly focused on broad germplasm collections, and side-by-side evaluations that align detailed morphological profiles with SSR patterns within well-defined mutant genotypes remain rare. This gap limits how breeders connect agronomically meaningful traits to genetic differentiation, constraining parent choice and targeted utilization in tropical systems.

In this study, the mutant variants of *A. compressus* used were previously produced through gamma irradiation [14]. Based on this resource, morphological and SSR-based molecular characterization were integrated to enable rapid and accurate discrimination among mutant genotypes. Specifically, seven *A. compressus* genotypes were evaluated using complementary phenotypic measurements and SSR markers and compared clustering outcomes to test phenotype and genotype consistency. This work identifies genotypes with distinct turf-relevant trait profiles and supports parent selection and breeding utilization in tropical turfgrass improvement programs.

2. Materials and methods

2.1 Plant materials and experimental location

The experiment was conducted at the UPM-SATIRI Turf Lab, Faculty of Agriculture, Universiti Putra Malaysia (UPM), located at Serdang, Selangor, Malaysia (2°59'N, 101°44'E; <https://maps.app.goo.gl/kxNZweoB7KzmgKW88>). The growth evaluation trial was carried out from January to March 2023. During this period, the site experienced tropical climate conditions characterized by average daily temperatures ranging between 23.9°C and 33.9°C, monthly rainfall varying from 186 to 259 mm, and relative humidity levels consistently between 77% and 79%. A total of seven *A. compressus* genotypes were used in this study including

the original species *A. compressus* (A-0), along with six mutant variants, namely A-1, A-40, A-46, A-91, A-122, and A-D. Among these variants, A-1 and A-91 are visually characterized by their longer leaves and larger leaf area. In contrast, A-40, A-46, and A-122 exhibit a shorter size, darker leaves. A-D shows dwarf and no obvious stolons [15]. All plant materials were cultivated at UPM-SATIRI Turf Lab, rendering no permits or herbarium voucher specimen's requirement for this study. All mutant variants were developed from the original species *Axonopus compressus* through mutation breeding by Prof. Dato' Dr. Abdul Shukor Juraimi, Dr. Mohd Said Saad, and Assoc. Prof. Dr. Muhammad Saiful Ahmad-Hamdani at Malaysia Nuclear Agency. Briefly, the original *Axonopus compressus* individuals were exposed to gamma (γ) radiation at the dosages of 200, 400, 600, and 800 Gy. All survivors were split into a single node and regrown in pots until maturity (approximately 4 months) at UPM-SATIRI Turf Lab. The selection process continued until the 8th generation before these trials were done.

2.2 Experimental design

Experiments were performed using a randomized complete block design (RCBD) with 3 replications. All plants were established using a single 15 x 15 cm sod in plastic trays of 37 x 27 x 10 cm, filled with growth medium of sand and peat-grow (8:2 v/v). Plants were watered thrice daily in the first 2 weeks after establishment, followed by one daily beginning third week until the completion of experiment. Weeds were constantly pulled out by hand so that they didn't compete. Fertilization was repeated twice every two weeks to encourage healthy plant growth at the rate of 0.25kg N/100m²/month. Twelve weeks after planting, morphological traits such as: leaf length (LL), leaf width (LW), number of leaves per plant (NLP), plant height (PH), stem thickness (STH), stolon thickness (STOLH), stem internode length (SIL), stolon internode length (STIL), shoot fresh weight (SFW), shoot dry weight (SDW), root length (RL), root fresh weight (RFW), leaf color index (LC), shoot density (SD), and root volume (RV) were measured.

2.3 Determination of morphological parameters

Stem thickness, stolon thickness, stem internode length, stolon internode length, as well as leaf length and leaf width on fully expanded functional leaf at third-to-last node using were measured using a Vernier caliper. Plant height was measured from the soil surface to the tip of the tallest leaf blade using a ruler. The measurements for root length were conducted with a measuring tape. The number of leaves per plant was determined by manually counting all fully expanded leaves on each selected 10 plants. Young, unexpanded leaves and senescent or detached leaves were excluded. Shoot density, fresh and dry weight of shoot and root components were assessed using a randomly placed 100 cm² grid on the turf surface. All harvested plants were washed, segregated, and their fresh weight was measured. The harvested samples were then oven-dried at 65°C for 72 hours until a constant weight was achieved. Root volume was subsequently measured using a graduated cylinder method. TCM 500 NDVI Turf Color Meter (Spectrum® Technologies, Inc., USA) was used to assess turf color. Leaf color was rated on a 1 to 9 scale: 1 meant withered grass or bare soil, whereas scores of 5 through 9 denoted yellow-green to dark green coloration.

2.4 DNA extraction and quantification

DNA was isolated using the CTAB extraction method (Doyle & Doyle, 1987). After cutting out leaf veins, around 0.1 g of young leaf tissue was ground with liquid nitrogen to a fine powder in a mortar and pestle. The homogenous powder was immediately placed into 2 mL microcentrifuge tubes. Then 700 μ L of CTAB extraction buffer was added to each tube. Samples were incubated at 65°C (10 min, 500 rpm) in a thermomixer, then put on ice for 10 min. Chloroform (800 μ L) was added and mixed gently before centrifugation (14,000 rpm, 20 min). The upper aqueous layer (750 μ L) was transferred to 1.5 mL microcentrifuge tube, followed by addition of cool isopropanol (650 μ L) to mixed. The sample was incubated at - 20°C for 20 minutes. Then, it was centrifuged at 14,000 rpm for 10 minutes. The supernatant was discarded, and the DNA pellet was washed three times with 200 μ L of 70% ethanol. After each washing, the sample was centrifuged at 14,000 rpm for 5 minutes. The final supernatant was removed, and the pellet was vacuum-dried for 10 minutes. Finally, the DNA was resuspended in 50 μ L of deionized water. DNA samples were initially quantified using a NanoDrop spectrophotometer (ND2000C), which provided estimates of DNA concentration and relative purity. Qualified samples were diluted to 50 ng/ μ L with nuclease-free water. All DNA extracts were stored at - 80°C for subsequent PCR analysis.

2.5 SSR primer screening and DNA amplification

A total of 10 pairs of SSR primers (Integrated DNA Technologies (IDT), USA) were selected and used for genetic diversity analysis [16]. The primer sequences and annealing temperatures are listed in Table 3. The 15 µL PCR reaction contained 7.5 µL of 2×PCR master mix, 1 µL of forward primer (10 µM), 1 µL of reverse primer (10 µM), 1 µL of genomic DNA template (50 ng/µL), and 4.5 µL of nuclease-free water. PCR amplification was performed using a touchdown protocol. The initial denaturation was carried out at 94°C for 5 min, followed by 24 cycles in the touchdown phase, starting with an annealing temperature of 60°C and decreasing by 0.5°C per cycle until reaching 48°C. Each cycle consisted of denaturation at 94°C for 45 s, annealing at primer-specific temperatures ($T_m \pm 5^\circ\text{C}$) for 45 s, and extension at 72°C for 1 min. A final extension was performed at 72°C for 7 min, after which the reactions were held at 4°C. SSR-amplified DNA products were separated on a 2.0% agarose gels. For each sample, 15.0 µL of PCR product was mixed with 3.0 µL of 6× loading dye, and a total volume of 18.0 µL was loaded into each well. A medium-range DNA ladder (100 bp, Integrated DNA Technologies (IDT), USA) was used as a size reference. Electrophoresis was performed at 150 V, and the amplified products were visualized under UV transillumination and documented using a gel documentation system (Biovis).

2.6 Data analysis

All analyses were performed in RStudio version 4.3.1. Morphological traits were analyzed by one-way ANOVA ($n = 3$), followed by Fisher's LSD at $P < 0.05$. Trait means of each variant were min–max normalized and used to generate a heatmap with hierarchical clustering (Euclidean distance; complete linkage) in the pheatmap package. SSR bands were scored as presence/absence (1/0), and monomorphic loci were removed. Jaccard distances were computed with the vegan package and used for UPGMA clustering with average linkage. PIC for dominant bands was calculated as described by Hasan et al. [17].

3. Results

3.1 Morphological differences among variants

The *A. compressus* variants evaluated in this study are shown in Figure 1. Significant differences were observed among variants for all measured traits (Table 1). Leaf morphology (LL, LW, LC), plant architecture (PH, STH, STOLH, SIL, STIL), and shoot growth traits (SD, SFW, SDW) all differed highly significantly among variants ($P < 0.001$). Root-related traits (RV, RL, RFW) and RNPLS also showed significant differences ($P < 0.05$ to $P < 0.001$). The coefficient of variation (CV) ranged from 1.62% to 59.73% (Table 2). The highest phenotypic variation was observed in shoot fresh weight (SFW) at 59.73%, whereas the lowest variation was observed in leaf color (LC) at 1.62%. Notably, the CVs of nine traits exceeded 30%.

Table 1. Comparative analysis of morphological traits in seven *A. compressus* variants using one-way ANOVA.

Variants	LW	LL	NLPS	PH	STH	STOLH	SIL	
A-0	9.39 ± 0.20 ^c	43.06 ± 2.98 ^c	43.17 ± 3.40 ^{bcd}	85.01 ± 3.10 ^{ab}	1.17 ± 0.10 ^{bc}	1.26 ± 0.04 ^b	17.21 ± 1.24 ^b	
A-1	11.18 ± 0.19 ^b	56.53 ± 4.13 ^a	35.25 ± 1.82 ^d	96.80 ± 5.49 ^a	1.32 ± 0.09 ^b	1.56 ± 0.05 ^a	21.97 ± 1.02 ^a	
A-40	6.96 ± 0.22 ^e	26.58 ± 1.17 ^d	59.42 ± 9.01 ^a	60.13 ± 5.69 ^{cd}	0.86 ± 0.04 ^d	1.04 ± 0.03 ^{cd}	13.51 ± 0.98 ^c	
A-46	7.97 ± 0.16 ^d	48.24 ± 3.67 ^{bc}	51.08 ± 2.96 ^{abc}	79.58 ± 4.59 ^b	1.04 ± 0.06 ^{cd}	1.14 ± 0.03 ^c	13.89 ± 1.06 ^c	
A-91	10.98 ± 0.37 ^b	52.84 ± 2.54 ^{ab}	42.92 ± 2.83 ^{bcd}	66.68 ± 3.95 ^c	1.37 ± 0.05 ^b	1.65 ± 0.04 ^a	16.72 ± 1.16 ^b	
A-122	5.74 ± 0.13 ^f	22.25 ± 1.15 ^d	52.92 ± 3.83 ^{ab}	47.90 ± 2.82 ^d	0.84 ± 0.03 ^d	0.94 ± 0.03 ^d	12.80 ± 0.87 ^c	
A-D	12.92 ± 0.34 ^a	28.36 ± 1.12 ^d	39.50 ± 3.09 ^{cd}	33.29 ± 4.11 ^e	3.03 ± 0.12 ^a	0.00 ± 0.00 ^e	4.11 ± 0.18 ^d	
P	***	***	**	***	***	***	***	
Variants	STIL	SFW	SDW	RL	RFW	LC	SD	RV
A-0	21.35 ± 1.04 ^{cd}	1.39 ± 0.22 ^b	0.42 ± 0.04 ^b	14.42 ± 1.42 ^b	0.31 ± 0.12 ^{ab}	6.26 ± 0.02 ^a	132.67 ± 6.17 ^{ab}	6.07 ± 0.30 ^b
A-1	32.60 ± 1.62 ^a	1.02 ± 0.13 ^{bcd}	0.45 ± 0.07 ^{ab}	15.25 ± 1.00 ^b	0.18 ± 0.03 ^{bc}	6.27 ± 0.01 ^a	129.67 ± 2.40 ^{ab}	13.50 ± 0.85 ^a
A-40	22.43 ± 1.31 ^{bc}	1.15 ± 0.18 ^{bc}	0.41 ± 0.03 ^b	13.73 ± 0.87 ^b	0.19 ± 0.05 ^{bc}	6.26 ± 0.01 ^a	163.33 ± 18.10 ^a	5.70 ± 0.61 ^{bc}
A-46	19.60 ± 1.22 ^{cd}	0.71 ± 0.09 ^{cd}	0.31 ± 0.03 ^{bc}	14.13 ± 0.77 ^b	0.18 ± 0.04 ^{bc}	6.16 ± 0.02 ^b	115.67 ± 14.80 ^b	7.33 ± 0.88 ^b
A-91	25.51 ± 1.55 ^b	1.04 ± 0.15 ^{bcd}	0.33 ± 0.04 ^{bc}	17.14 ± 1.25 ^{ab}	0.22 ± 0.04 ^{bc}	6.19 ± 0.02 ^{ab}	68.33 ± 4.33 ^c	11.93 ± 1.91 ^a
A-122	18.43 ± 0.72 ^d	0.48 ± 0.07 ^d	0.19 ± 0.02 ^c	13.83 ± 1.30 ^b	0.12 ± 0.02 ^c	6.08 ± 0.03 ^c	140.67 ± 17.70 ^{ab}	2.53 ± 0.52 ^c
A-D	0.00 ± 0.00 ^e	2.72 ± 0.41 ^a	0.57 ± 0.09 ^a	20.58 ± 2.38 ^a	0.43 ± 0.09 ^a	6.01 ± 0.06 ^c	64.67 ± 6.17 ^c	6.50 ± 1.44 ^b
P	***	***	***	**	*	***	***	***

Values are expressed as means ± standard error (n = 3); different letters within a column indicate significant differences at P < 0.05 according to Fisher's LSD test. Abbreviations: LL = Leaf length; LW = Leaf width; NLPS = Number of leaves per shoot; PH = Plant height; STH = Stem thickness; STOLH = Stolon thickness; SIL = Stem internode length; STIL = Stolon internode length; SFW = Shoot fresh weight; SDW = Shoot dry weight; RL = Root length; RFW = Root fresh weight; LC = Leaf color index; SD = Shoot density; RV = Root volume. Units: LL, LW, PH, STH, STOLH, SIL, STIL – mm; RL – cm; SFW, SDW, RFW – g; RV – cm³; LC – index (measured using a Turf Color Meter); NLPS – count (leaves per plant); SD – shoots/m² or visual rating (specified in the text, if applicable). *P < 0.05; **P < 0.01; ***P < 0.001.

Table 2. Descriptive statistics of morphological traits among the seven *A. compressus* variants.

Traits	Mean	SD	Min	Max	CV (%)
LL (mm)	39.69	13.82	22.25	56.53	34.81%
LW (mm)	9.31	2.56	5.74	12.92	27.53%
NLPS (No.)	46.32	8.45	35.25	59.42	18.24%
PH (mm)	67.06	22.06	96.80	33.29	32.89%
STH (mm)	1.38	0.76	3.03	0.84	55.10%
STIL (mm)	14.32	5.48	21.97	4.11	38.26%
STOLH (mm)	1.27	0.29	1.65	0.94	22.56%
STIL (mm)	23.32	5.16	32.60	18.43	22.14%
SD (No. shoot / 100 cm ²)	116.43	37.01	163.33	64.67	31.79%
RV (cm ³)	7.65	3.80	13.50	2.53	49.62%
RL (cm)	15.58	2.50	20.58	13.73	16.04%
SFW (g)	1.22	0.73	2.72	0.48	59.73%
SDW (g)	0.38	0.12	0.57	0.19	31.44%
RFW (g)	0.23	0.10	0.43	0.12	44.74%
LC (index)	6.18	0.10	6.27	6.01	1.62%

3.2 Representative morphological types

The heatmap based on normalized quantitative traits grouped the seven *A. compressus* variants into three clusters (Figure 2). Cluster I (A-D and A-40) showed high biomass-related trait means, with high shoot fresh and dry weight and the longest roots. Cluster II (A-122, A-0, and A-46) exhibited lower biomass but the highest shoot density. Cluster III (A-1 and A-91) was characterized by the largest plant size, with the greatest leaf width and length, tallest plants, and largest root volume, although with the lowest shoot density.

A-1 exhibited the tallest and largest phenotype among all variants, including the greatest leaf length (56.53 mm), plant height (96.80 mm), stolon internode length (32.60 mm) combined with the most extensive root system. A-40 had the maximum leaf number per plant (59.42), with relatively smaller leaves, high leaf color intensity, and a moderate plant height (60.13 mm). A-D was a dwarf phenotype and lacked stolon development. It showed the greatest leaf width (12.92 mm), maximum stem thickness, and longest root length (20.58 cm). A-D also showed the highest shoot fresh weight (2.72 g).

3.3 SSR primer polymorphism analysis

A total of 10 SSR primer pairs were initially screened, with eight successfully amplifying DNA fragments. Among these, six primer pairs with high polymorphism information content (PIC) values (IDs: 94, 87, 73, 67, 34, and 25) were selected for further polymorphic analysis (Table 3). These primers produced fragments ranging in size from 100 bp to 350 bp (Table 3; Figure 3), with an average polymorphic information content (PIC) of 0.357. Genetic divergence among the variants was evaluated using the Jaccard distance matrix (Table 4). The genetic distance values ranged from 0.200 to 0.857. The highest distance was recorded between the original A-0 and A-91 (0.857), indicating the most significant genetic divergence. The lowest genetic distance was observed between A-46 and A-D (0.200), suggesting that these two genotypes share the closest genetic relationship. A-0 showed different degrees of divergence from the variants, with distances ranging from 0.500 (with A-1 and A-46) to 0.857 (with A-91).

Table 3. Six pairs of selected SSR primers and their amplification profiles used in this study.

SI	Locus	Forward Primer Sequence	Reverse Primer Sequence	Ta (°C)	Size (bp)	AB	PB	PIC
1	ID25	AGCATCGTCGAAAAACCTGT	TTGCATGAAAGTAAAGCAATGAA	55	200	2	1	0.408
2	ID34	TGCTTGGCCTCTAGCCTACT	ACCAGCAGATGTGGTTGATT	58	100-200	2	1	0.245
3	ID67	TGAAGTCAATTAGGATTTTATGGG	TGCGAGATGAGTTCGAGTATC	52	100-350	2	2	0.571
4	ID73	TTCCCCACTAAAAATGACGG	CAATCTTATCCGCCATGAAA	50	150	2	1	0.501
5	ID87	AGGGGGCAGCTCATTTTTAT	ATTCAGGACTCGGTTGATGC	56	200	1	1	0.489
6	ID94	GGCCATATAAGGTGACGCAT	TTTTCATGGTTGCCAAATCA	55	150-250	2	2	0.245

Note: Amplified bands-AB; Polymorphic bands-PB; PIC-Polymorphism Information Content

Table 4. Pairwise Jaccard distance matrix based on polymorphic SSR loci among seven *A. compressus* variants.

Sample	A-0	A-122	A-1	A-46	A-40	A-91
A-122	0.750					
A-1	0.500	0.429				
A-46	0.500	0.429	0.333			
A-40	0.800	0.667	0.600	0.600		
A-91	0.857	0.333	0.500	0.500	0.500	
A-D	0.667	0.571	0.500	0.200	0.800	0.667

3.4 Genetic relationships among varieties

The genetic relationships among the seven variants were illustrated through a UPGMA dendrogram based on Jaccard distance coefficients (Figure 4). At a genetic distance of 0.5, the seven *A. compressus* variants were separated into three major clusters: Cluster I consisted of A-D, A-46, A-1, A-91, and A-122; Cluster II consisted of A-40; and Cluster III consisted of A-0. In addition, PCoA based on Jaccard distances explained 75.0% of the variation on the first principal coordinates (PCoA1: 46.0%; PCoA2: 29.0%) (Figure 5). Notably, A-0 was positioned at the positive extreme of PCoA1, clearly separated from all mutant lines. Similarly, pairs that were close in the dendrogram (A-46 and A-D; A-91 and A-122) also occupied nearby coordinates in the PCoA plot.

4. Discussion

The objectives of turfgrass breeding are generally directed toward improving aesthetic appearance and enhancing resistance to environmental stress. Mutation breeding is a method for improving turfgrass and generating new variations [18]. As a traditional method for phenotypic identification of germplasm resources, morphological assessment still plays an indispensable role in breeding and germplasm conservation, especially in resource screening and variation analysis [19]. When combined with molecular markers methods, it can further improve evaluation accuracy and resolution [17, 20].

Morphological characterization is suitable for preliminary classification of turfgrass germplasm resources and the identification of desirable traits [21, 22]. The observed differences among *A. compressus* varieties indicate significant phenotypic variability. The coefficients of variation (CV) values varied widely across traits, ranging from 1.62% to 59.73%. Among the measured traits, shoot fresh weight showed the highest variability (CV = 59.73%), suggesting that biomass-related traits are effective for differentiating these variants phenotypically [23, 24]. Compared to A-0, A-D showed a dwarf phenotype

with reduced leaf size and diameter but the longest roots. These traits may be useful in selection when belowground performance is a priority [25, 26]. A strong root system is often associated with improved drought tolerance and water-use efficiency [27].

A similar pattern has been reported in Bermuda grass and Orchard grass, where dwarf genotypes compensate for aboveground competition by increasing root development [28, 29]. The high leaf number per shoot, increased shoot density, and darker leaf colour of A-40 can enhance photosynthetic efficiency and promote a denser canopy [30]. In addition to A-40, A-122 and A-46 exhibited similar compact growth traits. A-1 and A-91 had greater leaf length and plant height, suggesting potential use where rapid establishment or biomass production is prioritized. Overall, mutagenesis can generate turfgrass mutants with desirable traits such as compact growth, improved canopy characteristics, and altered root traits. Similar improvements have been reported in radiation-induced mutants of St. Augustine grass [31], Ryegrass [32], and *Zoysia* spp. [33], which exhibited prostrate and dwarf habits as well as improved mowing tolerance and root traits. These studies support the value of induced morphological variation for turfgrass improvement.

SSR markers are also useful in detecting genetic differences among turfgrass variants due to their high polymorphism and ability to discriminate morphologically similar genotypes [34, 35], as well as in parent selection and variety identification in breeding programs [36]. In this study, SSR analysis revealed substantial genetic differentiation, with Jaccard distances ranging from 0.200 to 0.857. The observed genetic differentiation is consistent with radiation-induced variation and may explain the development of distinct phenotypes, such as dwarf habit of A-D. However, the underlying mutational mechanisms require further investigation. Although the average PIC value of 0.357 is moderate, such values are common in turfgrass and other vegetatively propagated species [37]. Further studies using more highly polymorphic markers, such as EST-SSRs or SNPs, may provide better resolution in genetic diversity analysis.

The grouping results based on morphological traits and SSR markers showed partial agreement. For example, morphology-based clustering grouped A-1 and A-91 together, whereas SSR-based clustering grouped A-D and A-46 together. This partial correspondence suggests that morphological similarity may not always fully reflect underlying genetic relationships [38, 39]. The observed discrepancies may reflect the distinction between functional traits and neutral genetic markers. Morphological traits can be influenced by environmental conditions and management practices, whereas SSR markers reflect variation at the DNA level [40]. The morphological similarity between A-40 and A-D, despite their relatively large genetic distance, suggests a case of phenotypic convergence, where different genetic pathways may have led to similar trait patterns [41]. In addition, variation in a few key regulatory genes might substantially impact phenotypic expression without affecting the majority of the genome [42]. Overall, the combination of morphological and molecular methods provided complementary perspectives, thereby improving the robustness of accession classification. Furthermore, the PCoA analysis, which explained 75.0% of the total variation, demonstrated that the SSR markers effectively captured the principal genetic differences among variants. Morphological and SSR-based clustering assessments revealed several accessions with potential breeding value. A-1 and A-91 had the largest leaf length, plant height, and root volume, indicating vigorous vegetative growth and strong establishment potential. A-40, A-122 and A-46 exhibited dense foliage and a high number of leaves, which may be advantageous for weed suppression and improved visual quality. A-D, with its dwarf growth habit, may be particularly suitable as a groundcover type. These accessions represent valuable germplasm resources for improving *A. compressus*. The high level of genetic differentiation among variants also provides a broad genetic base that could be exploited in hybrid breeding programs.

5. Conclusion

This study evaluated morphological variation and SSR-based genetic diversity among seven *A. compressus* variants. The results showed that gamma irradiation generated significant phenotypic and genetic differentiation. The morphological and molecular data provide complementary information for germplasm identification and parental selection. These results enhance the understanding of *A. compressus* diversity and support accurate germplasm identification and targeted parent selection for future breeding programs.

Declarations

Author Contributions

T.Z., M.S.A.-H., A.S.J., and N.S.N. designed the experiments. M.S.A.-H., A.S.J., and N.S.N. supervised the research and provided guidance on data analysis and interpretation. T.Z. conducted the experiments, performed data analysis, and wrote the original manuscript. R.R. assisted with the experimental work. D.L. contributed to data interpretation. M.S.A.-H. and R. R. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability

The datasets supporting the conclusions of this article are included within the article (and its additional files).

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

(a)



(b)

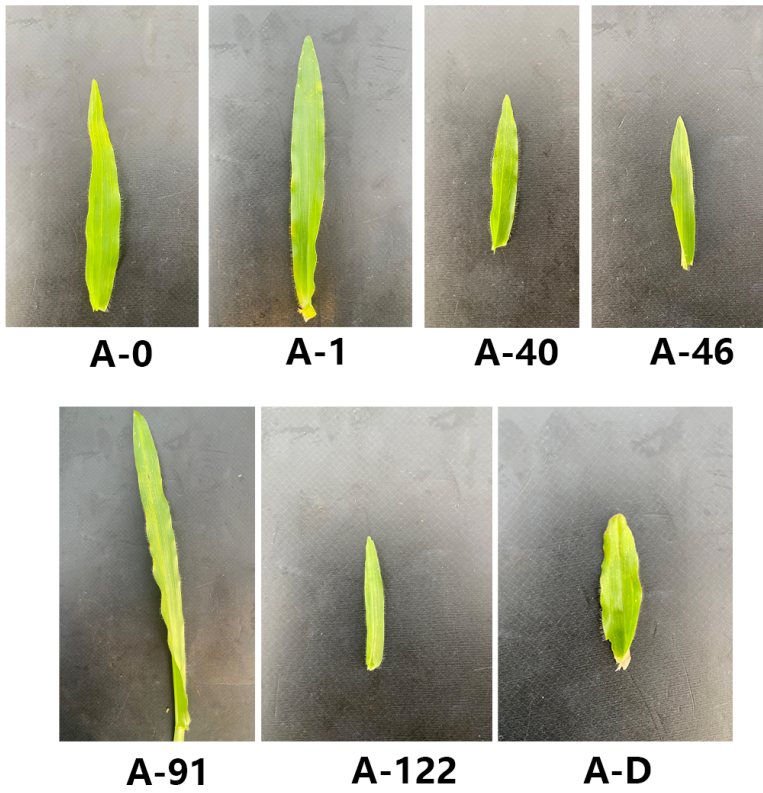


Figure 1

Morphological characteristics of seven *A. compressus* variants. (a) Whole-plant morphology, including shoots and roots; (b) representative leaf shape of each variant.

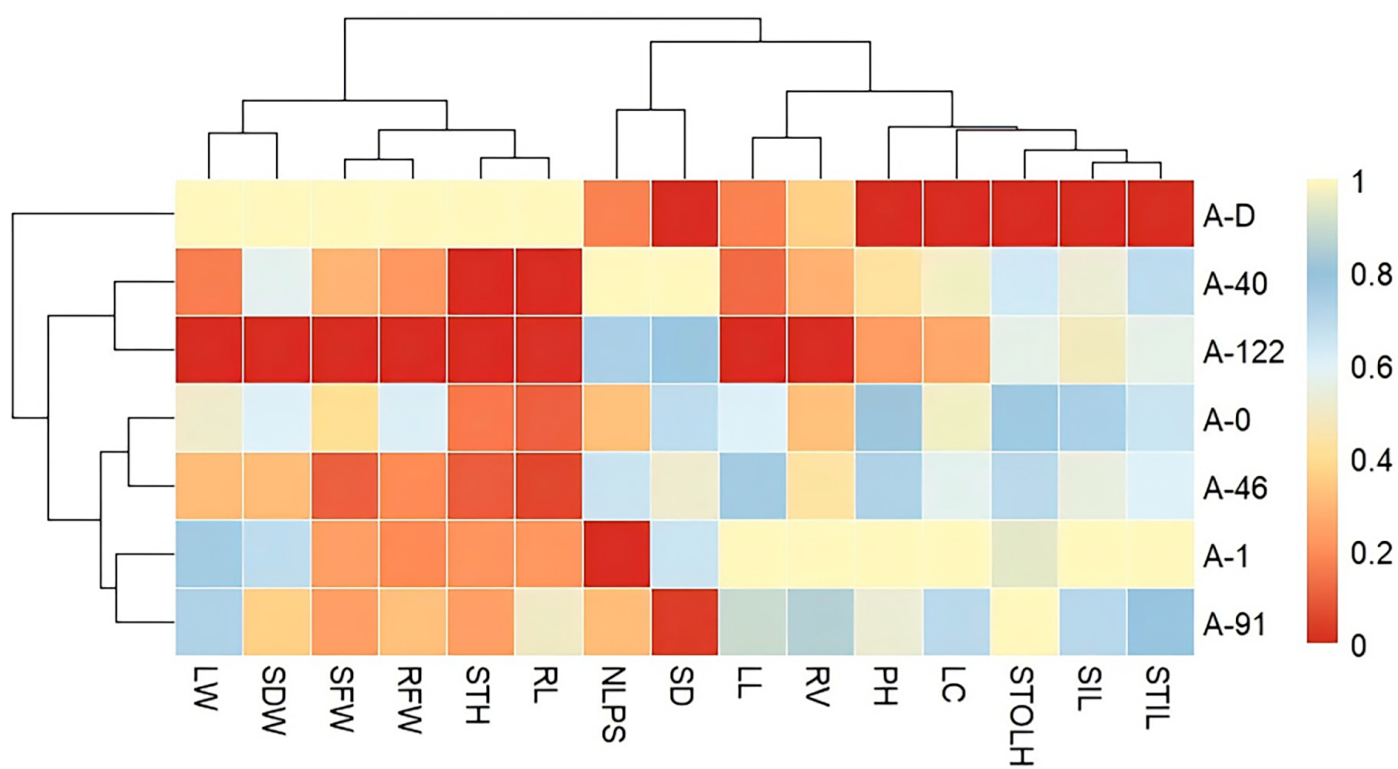
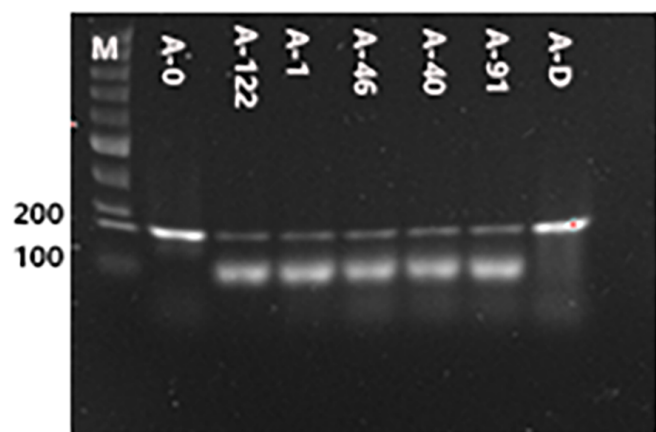
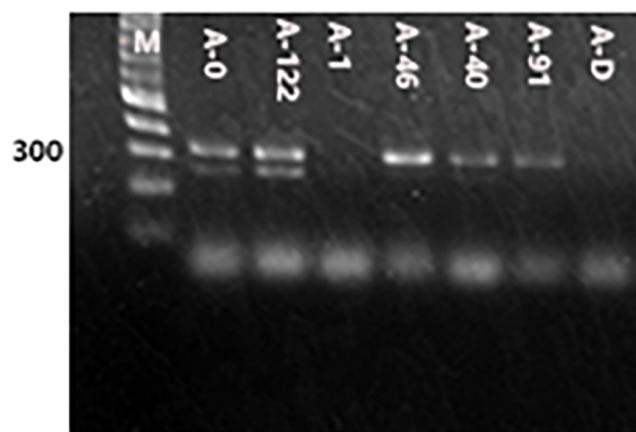


Figure 2

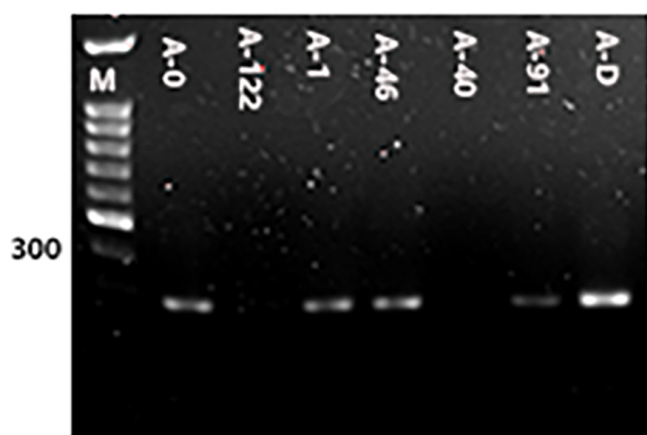
Heatmap of min-max normalized quantitative traits of seven *A. compressus* variants, with hierarchical clustering based on Euclidean distance (complete linkage).



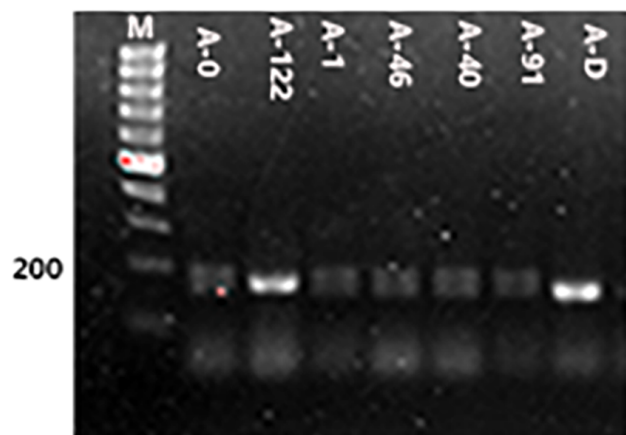
(a)



(b)



(c)



(d)

Figure 3

DNA fingerprinting pattern generated using four SSR markers: (a) SSR ID34; (b) SSR ID94; (c) SSR ID 87; (d) SSR ID73.

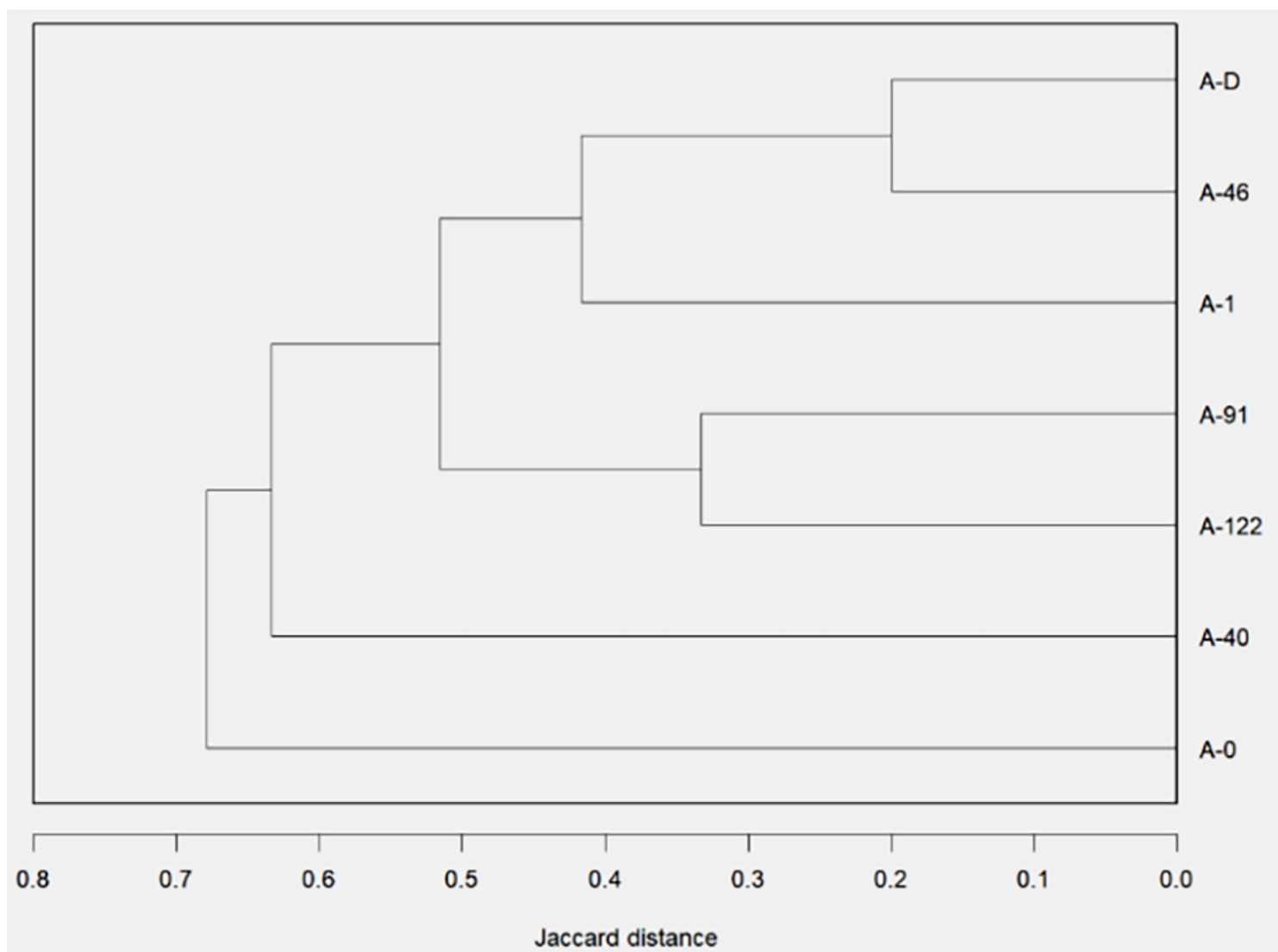


Figure 4

UPGMA cluster dendrogram of *A. compressus* variants based on SSR markers using Jaccard's similarity coefficient.

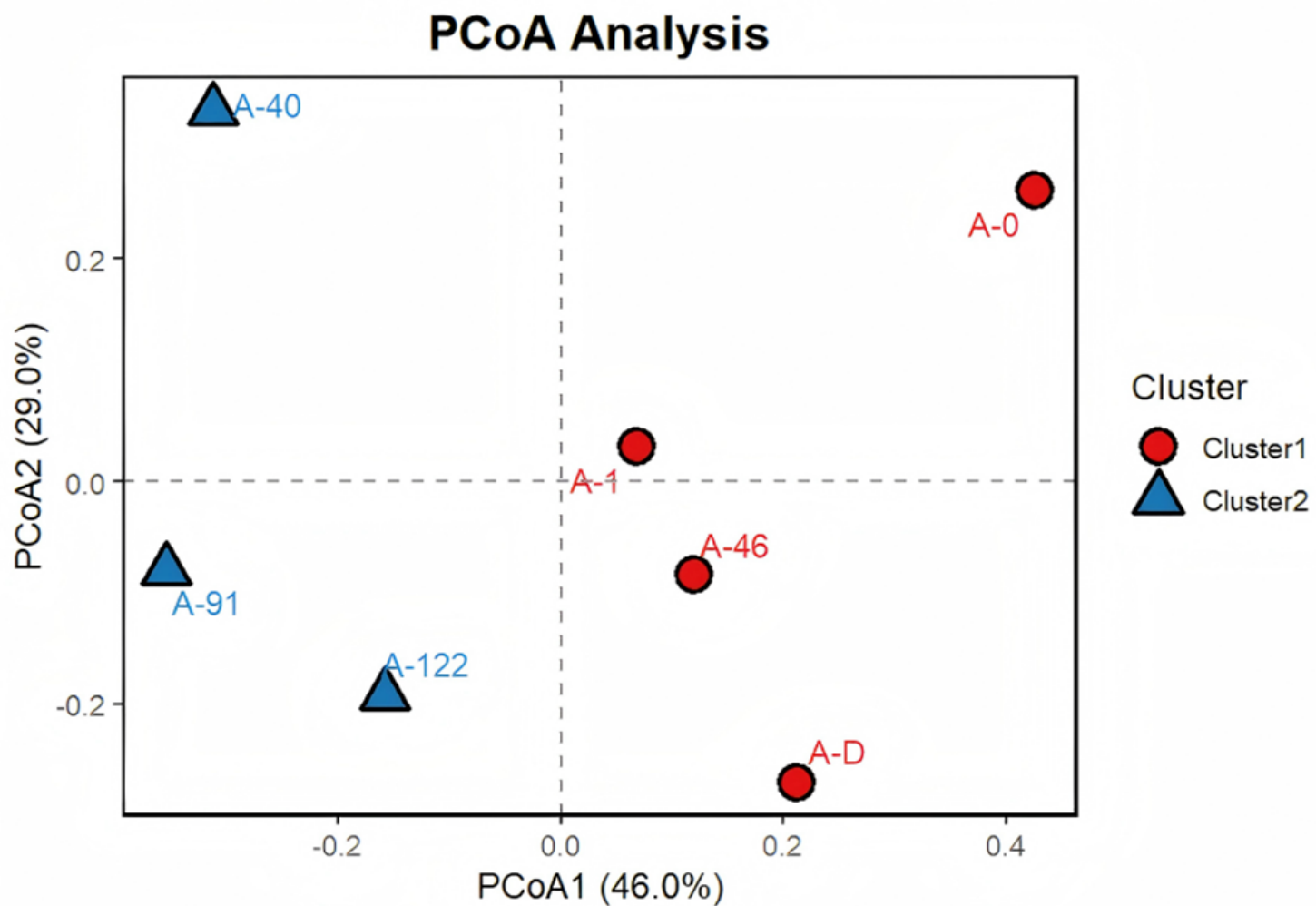


Figure 5

Principal coordinate analysis (PCoA) of the seven *A. compressus* variants based on SSR data.