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Assessment of Eight Faba Bean (*Vicia faba* L.) Cultivars for Drought Stress Tolerance through Molecular, Morphological, and Physiochemical Parameters

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Abstract: Determining and improving drought-tolerant cultivars is a major goal of plant breeding to face climate change. The productivity of faba bean in Egypt is affected by abiotic stresses, especially drought stress. This study evaluated eight Egyptian faba bean cultivars for drought tolerance under three soil water regimes consisting of well-watered (100% field capacity), moderate drought stress (50% field capacity), and severe drought stress (25% field capacity) regimes in pots under greenhouse conditions using biochemical, physiochemical, and molecular parameters. The cultivars Nubariya 1, Nubariya 3, and Giza 716 showed the highest proline content values under 50% field capacity conditions, with 4.94, 4.39, and 4.26 mmol/g fresh weights, respectively. On the other hand, the cultivars Sakha 1, Sakha 4, Nubariya 1, and Nubariya 3 exhibited the highest proline contents (7.8, 7.53, 6.17, and 6.25, respectively) under 25% field capacity treatment. The molecular profiling was conducted using SCoT and SRAP approaches. Fresh leaves were utilized to extract the DNA, and ten primers for SRAP and six for SCoT were used in the PCR procedures. SCoT and SRAP-PCR generated 72 loci, of which, 55 were polymorphic, and 17 were monomorphic. SCoT and SRAP each had 48 and 24 total loci, respectively. The average polymorphism (%) values achieved via SCoT and SRAP were 70.93% and 80%, respectively. Based on the molecular profiles, the cluster analysis identified three clusters. The first cluster comprised Giza 716 cultivars; the second cluster included Sakha 1, Sakha 3, Sakha 4, and Akba 3300 cultivars; the third cluster comprised two cultivars Nubariya 1 and Nubariya3. According to the study's findings, Sakha 1, Sakha 4, Nubariya 1, and Nubariya 3 are remarkable parents for developing drought-tolerant faba bean genotypes. Additionally, this study concluded that SRAP and SCoT markers recreated trustworthy banding profiles to evaluate the genetic polymorphism among faba bean cultivars, which are regarded as the cornerstone for genetic improvements in crops.

Keywords: faba bean; drought tolerance; molecular markers; SCoT; SRAP; abiotic stress

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1. Introduction

The faba bean (*Vicia faba* L. 2*n* = 12) is regarded as a critical legume crop used in human and animal nutrition due to its high protein content (20–30%) [1–3]. Most faba bean cultivars are susceptible to abiotic stress. A lack of appropriate genetic background and reasonable tolerance to environmental stresses are the main causes of the yield's instability. The primary objectives of faba bean breeding programs are high yield and tolerance to stresses [4].

Generating cultivars that are adapted to the environmental conditions in which faba bean is cultivated is the most effective strategy to overcome the abiotic stresses of faba

bean production [5]. It is undeniable that genetic variations created through mutations or hybridizations enable the selection of genotypes adapted to environmental factors such as drought, temperature, and salt soil, or genotypes resistant to pests and diseases [6].

Drought stress affects plant organ growth by altering the morphological and physiological features of plants [7]. The mechanism involved in adapting plants to drought is variation in the ratio of root/shoot dry mass [8,9]. Drought stress results in growth reduction and decreases the growth of the shoot and root of bean plants. This reduction may be due to decreased photosynthesis, the growth of the plant, expansion, and the division of plant cells [10,11].

Various molecular markers have been utilized to demonstrate the genetic variation in plants and other organisms [12–14]. Despite having a long history of usage, morphological and biological markers have certain disadvantages, including susceptibility to environmental variables [15]. As a result, various DNA markers have been developed, including SSR, RFLP, RAPD, and AFLP [16,17]. Molecular markers are fast, unaffected by environmental conditions, and reliable for selecting important agricultural characteristics [18]. As a result, they have been used to detect genetic polymorphism in faba bean plants. SSR markers, also known as microsatellites, have been used in many crops because they are highly polymorphic, based on PCR, and easily transferable.

Numerous co-dominant markers are revealed by the sequence-related amplified polymorphism (SRAP) marker, which is also more repeatable than RAPDs, easier to test than AFLPs, and, most importantly, targets open reading frames (ORFs). The SRAP-PCR-based system is a dominant marker technique, simple, inexpensive, and effective for producing genome-wide fragments with high reproducibility and versatility [19]. This marker was originally developed for gene tagging in *Brassica oleracea* L. to specifically amplify coding regions of the genome with ambiguous primers targeting GC-rich exons (forward primers) and AT-rich promoters, introns, and spacers (reverse primers). It is an effective and simple molecular marker approach [19]. It was employed to assess the genetic variation of legumes [20].

A recent technique called start codon targeted (SCoT) was generated to start a trend away from random DNA markers and toward gene-targeted markers based on the short conserved region flanking the ATG of plant genes. Since the SCoT marker is often trustworthy, it is recognized that annealing temperature and primer length are not the only variables affecting reproducibility [21].

Using morphological and physiochemical parameters, this study attempted to assess the field performance of eight faba bean cultivars under drought conditions. It also used SCoT and SRAP molecular markers to assess the genetic variation levels among used faba bean cultivars.

2. Materials and Methods

2.1. Plant Materials and Field Experiment

Eight faba bean cultivars, Nubariya 1 (Egypt), Nubariya 3 (Egypt), Giza 716 (Egypt), Giza 843 (Egypt), Sakha 1 (Egypt), Sakha 3 (Egypt), Sakha4 (Egypt), and Akba 3300 (Sudan), were used in this study. The plants were grown in pots in a greenhouse with natural light at a temperature of 25 °C and 15 °C during the day and the night, respectively.

The seeds of each cultivar were grown in pots filled with 5 kg of soil under three soil water regimes consisting of a well-watered (100% field capacity), moderate drought stress (50% field capacity), and severe drought stress (25% field capacity) regime. The field capacity was measured according to the method of Sarkar [22]. The experiment was conducted in 3 replicates using Complete Randomized Block Design (CRBD). Each replicate included five plants of each cultivar.

2.2. Drought Tolerance Parameters

Morphological Measurements

The number of leaves was measured after 30 days from sowing. At 60 days, the shoot length, root length, shoot dry weight (g), and root dry weight were recorded as morphological parameters for drought tolerance.

2.3. Proline Content Determination

The content of proline in leaf samples was measured as a physiochemical parameter for selecting drought-resistant genotypes according to Bates et al. [23]. In total, 0.5 g of leaves was blended in 10 mL of aqueous sulfosalicylic acid (3%), followed by filtration through filter paper. A test tube containing 2 mL of filtrated samples, 2 mL of acid-ninhydrin, and 2 mL of glacial acetic acid was heated to 100 °C for 1 h, and the tube was subjected to ice to stop the reaction. Four ml of toluene was used to extract the reaction mixture and stirred for 20 s. Toluene was used as a blank to measure the absorbance of the chromophore after it was aspirated and warmed to room temperature. The proline concentration was computed from the following equation:

$$\mu \text{ moles proline / g of fresh weight} = \frac{(\mu\text{g proline / mL} \times \text{mL toluene})}{[115.5\ \mu\text{g / } \mu\text{mole}]/[(\text{g sample})/5]} \quad (1)$$

2.4. Extraction of Genomic DNA

All genotypes of three-week-old faba bean leaves were utilized for the extraction of DNA, which was performed using the CTAB method with some modifications [24]. NanoDrop was utilized to evaluate the quantity and quality of total DNA purified.

2.5. Start Codon Targeted (SCoT) Amplification

A reaction of 25 µL volume of SCoT-PCR-based marker was conducted using 10 µL of GoTaq Green, 1 µL of template DNA, 1 µL of primer, Master Mix, and 25 µL of nuclease-free water. On a thermal cycler (applied biosystems), amplification was performed using the following program: 94 °C for 5 min (initial denaturation), then 30 cycles of 1 min each of denaturation, annealing, and elongation at 94 °C, 50 °C, and 72 °C, respectively.

2.6. Sequence-Related Amplified Polymorphism (SRAP) Amplification

A reaction of 25 µL volume of SRAP–PCR-based marker was conducted using 10 µL of the GoTaq Green Master Mix, 1 µL each of the forward and reverse primers (Table 1), 1 µL of the template DNA, and 25 µL of nuclease-free water. On a thermal cycler (applied biosystems), amplification was performed using the following program: 94 °C for 5 min (initial denaturation), then five cycles of one minute each of denaturation (94 °C), annealing (35 °C), and elongation (72 °C). This was followed by 30 cycles with an annealing temperature of 50 °C for 1 min and the final extension for 7 min at 72 °C.

Table 1. SRAP and SCoT primers with their nucleotide sequences, annealing temperature, Tm °C, molecular weight g/mol, primer length, and GC % content.

Primers		Sequences (5′-3′)	Tm (°C)	Molecular Weight g/mol	Primer Length	GC % Content
SRAP	ME1	TGAGTCCAAACCGGATA	57.6	5203.5	17	47.06
	ME2	TGAGTCCAAACCGGAGC	62	5204.4	17	58.82
	ME3	TGAGTCCAAACCGGAAT	58.6	5203.5	17	47.06
	ME4	TGA GTCCAAACCGGACC	61.7	5164.4	17	58.82
	ME5	TGAGTCCAAACCGGAAG	59.2	5228.5	17	52.94
	EM6	GACTGCGTACGAATTAAT	55.4	5522.7	18	38.89
	EM7	GACTGCGTACGAATTTGC	59.7	5514.7	18	50
	EM8	GACTGCGTACGAATTGAC	58.8	5523.7	18	50
	EM9	GACTGCGTACGAATTTGA	58.1	5538.7	18	44.44
	EM10	GACTGCGTACGAATTAAC	56.3	5507.7	18	44.44

Table 1. *Cont.*

Primers		Sequences (5′-3′)	Tm (°C)	Molecular Weight g/mol	Primer Length	GC % Content
SCoT	SCoT3	CAACAATGGCTACCACCC	61.2	5397.6	18	55.56
	SCoT4	ACCATGGCTACCACCGGC	67.6	5429.6	18	66.67
	SCoT5	CAACAATGGCTACCACGC	62.0	5437.6	18	55.56
	SCoT6	CAACAATGGCTACCACCG	61.4	5437.6	18	55.56
	SCoT7	ACGACATGGCGACCACGC	68.2	5478.6	18	66.67
	SCoT8	CCATGGCTACCACCGCAG	65.8	5429.6	18	66.67

2.7. Gel Electrophoresis

SCoT and SRAP-PCR-based banding profiles were visualized by 1% and 2% agarose gel, respectively. The agarose gel was stained in TBE buffer with ethidium bromide (pH 8.5). The final step involved using the gel documentation system to take pictures of the PCP products resulting in the presence of a 1 kbp DNA ladder as a molecular size reference (Goddard Irvine, CA, USA).

2.8. Data Analysis

The statistical program SPSS was used to examine the collected data. All measurements were recorded, and the significant differences among mean values at $p < 0.05$ were obtained by $L.S.D_{0.05}$ according to Snedecor et al. [25]. To identify differences among the examined cultivars, a two-way analysis of variance was utilized.

For each primer or primer combination, eight cultivars of the SCoT- and SRAP-amplified bands were assessed as present (1) or absent (0). According to Dice assessment, genetic similarity between cultivars was estimated [26] using the IBM SPSS statistical program [27]. The phylogeny analysis [28] was applied to group and generate the linkage dendrogram using the STATISTICA 8 program [29].

3. Results

3.1. Morphological and Physiochemical Parameters

Morphological and physiochemical parameters were recorded under normal irrigation and drought conditions with all studied cultivars to study the effect of drought treatments on the performance of cultivars compared to the normal irrigation regime. The averages of the studied traits for the studied cultivars over the morphological and proline content are presented in Figure 1. Overall, in the studied cultivars, significant differences were presented between the control, 50% field capacity, and 25% field capacity (Table 2). The Giza 843 cultivar showed the highest number of leaves at 30 days under the normal irrigation regime, while Akba 3300 showed the lowest number of leaves at 30 days under the same conditions. Under 50% field capacity, all cultivars presented a moderate reduction in the number of leaves. In the case of 25% field capacity, all cultivars showed no significant differences with 50% field capacity in the number of leaves at 30 days except the Giza 716 and Sakha 3 cultivars (Figure 1A). Clear significant differences appeared among all studied treatments for the length of shoots and the length of roots at 60 days, the dry weight of shoot (g), and the dry weight of root (Figure 1B–F). The relative mean decrease was found in most parameters with decreased field capacity except with proline content (Figure 1F).

In plants grown under drought conditions, the proline rises proportionately more quickly than other amino acids; for this reason, proline content is used to evaluate stressed plants. We selected proline to evaluate cultivars for the best recovery reaction the plant showed to face water shortage stress. Sakha 1 and Sakha 4 presented the best response by accumulating the highest proline content, indicating that they may be the best two cultivars that can recover under drought stress.

The analysis of variance for the studied parameters presented in Table 3 revealed that faba bean cultivars were significantly different in terms of the shoot length at 60 days (cm),

shoot dry weight at 60 days (g), and root dry weight at 60 days (g) parameters, while the differences were highly significant for the number of leaves at 30 days, root length at 60 days (cm), and proline content. The differences were significant among treatments for the number of leaves at 30 days and highly significant in the case of shoot length at 60 days (cm), root length at 60 days (cm), shoot dry weight at 60 days (g), root dry weight at 60 days (g), and proline content. The number of leaves at 30 days, shoot dry weight, and root dry weight at 60 days did not show a significant interaction between genotypes and treatment, indicating that environmental factors may significantly impact cultivar performance. For root length at 60 days and proline content, the interaction was highly significant and significant (Table 3).

Table 2. Effect of drought stress treatments on growth performance of faba bean cultivars.

Treatments	No. of Leaves/Plant at 30 Days	Shoot Length at 60 Days (cm)	Root Length at 60 Days (cm)	Shoot Dry Weight at 60 Days (g)	Root Dry Weight at 60 Days (g)	Proline Content
100% field capacity	5.9517 ^{a}	27.250 ^{a}	21.667 ^{a}	5.0654 ^{a}	4.6892 ^{a}	2.7964 ^{c}
50% field capacity	5.5167 ^{b}	18.958 ^{b}	13.708 ^{b}	4.5008 ^{b}	3.0313 ^{b}	3.6393 ^{b}
25% field capacity	4.9667 ^{c}	11.958 ^{c}	10.000 ^{c}	4.3187 ^{c}	2.8608 ^{c}	5.9700 ^{a}
$LSD_{0.05}$	0.4254	1.5329	1.2756	0.1723	0.1380	0.4073

Values within a column followed by the different letters are statistically different at $p < 0.05$.

Table 3. Analysis of variance for faba beans' studied traits under drought conditions.

Source of Variance S.O.V	Df	No. of Leaves/Plant at 30 Days	Shoot Length at 60 Days (cm)	Root Length at 60 Days (cm)	Shoot Dry Weight at 60 Days (g)	Root Dry Weight at 60 Days (g)	Proline Content
Replications	2	0.87500	1.26	3.167	0.04366	0.0587	106.850
Treatments	2	1.37897 *	1406.35 **	852.792 **	3.63768 **	24.4821 **	64.859 **
Cultivars	7	3.37500 **	12.86 *	32.236 **	0.23171 *	0.1338 *	2.261 **
Treatments × Cultivars	14	0.67659	14.84 *	12.760 **	0.08193	0.0511	2.964 **
Error	46	0.61413	6.96	4.819	0.08789	0.0564	0.491

Df is the degree of freedom, ** indicates p-value < 0.01, and * indicates p-value < 0.05.

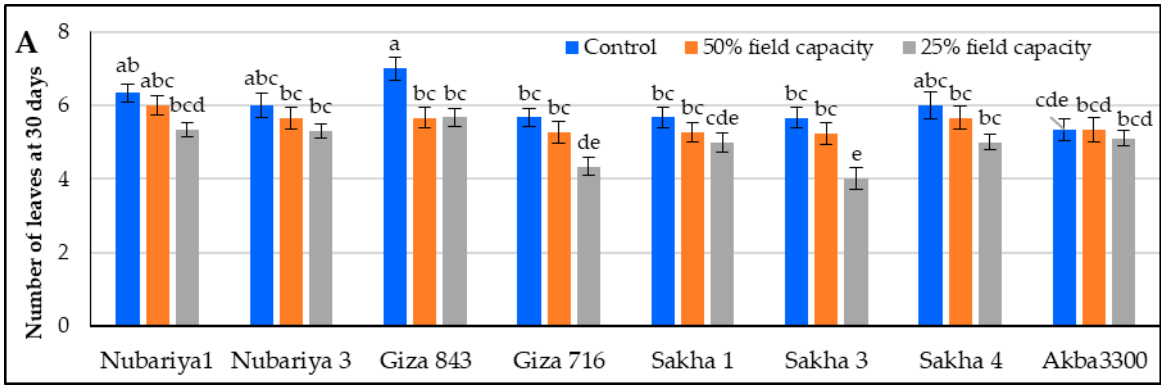
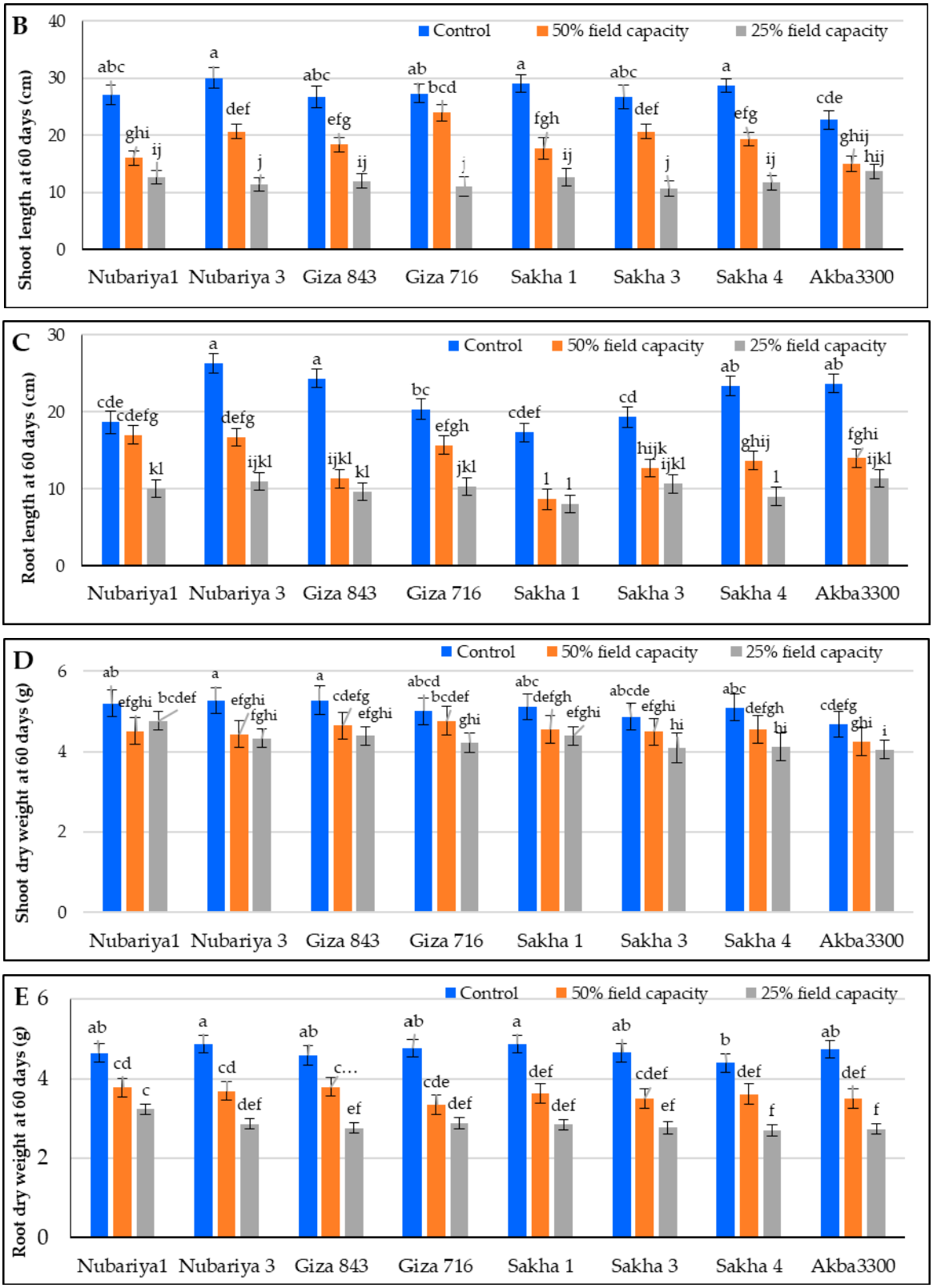


Figure 1. *Cont.*



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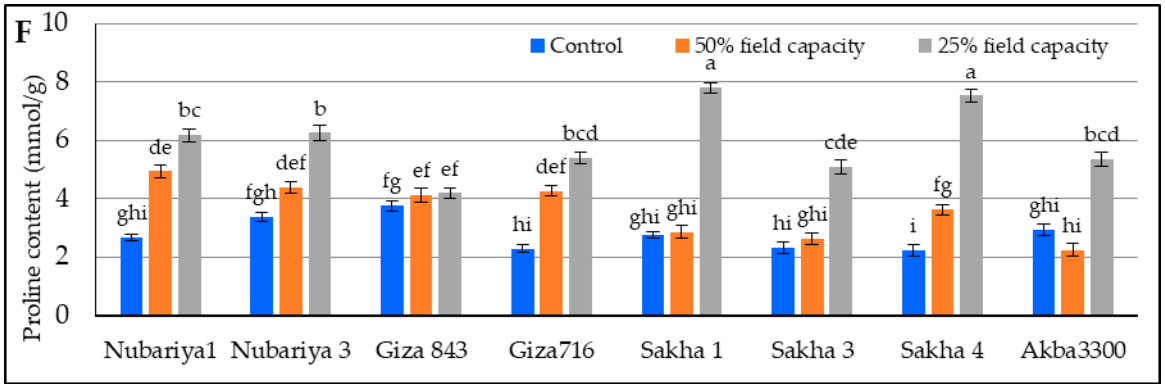

Figure 1. The performance of faba bean cultivars for studied traits under normal irrigation and drought stress treatments. (**A**). Number of leaves at 30 days. (**B**). Shoot length at 60 days (cm). (**C**). Root length at 60 days (cm). (**D**). Shoot dry weight at 60 days (g). (**E**). Root dry weight at 60 days (g). (**F**). Proline content. Note: Charts with variable letters are statistically different at $p > 0.05$.

3.2. Genetic Polymorphism Analyses

The molecular polymorphism analysis among the eight faba bean cultivars was assessed by SRAP and SCoT molecular markers using 16 primers (10 primers for SRAP-PCR reaction and 6 primers for SCoT-PCR reaction) (Section 2).

Forty-eight loci were observed using SCoT-PCR primers screened across eight cultivars (Figure 2 and Table 4). The number of amplified loci/primer ranged from 10 (SCoT8) to 6 (SCoT5), with an average of 8 loci per primer (Table 4). In SCoT-PCR reactions, out of the 48 amplified loci, 35 were polymorphic loci with an average mean of 5.83 polymorphic loci/primer. The percentage of polymorphism ranged from 100% (SCoT 8) to 50% (SCoT 5), with an average of 70.93% polymorphism.

Table 4. Number of bands, polymorphic, monomorphic, and unique, generated by SCoT and SRAP primers in eight faba bean cultivars and the related polymorphism.

PCR Type	Primers	Number of Bands	Monomorphic Bands	Polymorphic Bands	Unique Bands	Polymorphism (%)
SCoT	SCoT3	8	3	5	0	62.5%
	SCoT4	8	2	6	1	75%
	SCoT5	6	3	3	0	50%
	SCoT6	7	2	5	0	71.42%
	SCoT7	9	3	6	2	66.66%
	SCoT8	10	0	10	2	100%
	Total	48	13	35	5	
	Average	8	2.16	5.83	0.833	70.93%
SRAP	ME3/EM9	4	0	4	1	100%
	ME4/EM8	8	0	8	0	100%
	ME5/EM10	7	0	7	0	100%
	ME2/EM10	5	4	1	0	20%
	Total	24	4	20	1	
	Average	6	1	5	0.25	80%
Total number of loci		72	17	55	6	

Twenty-four loci were observed using SRAP-PCR primers screened across eight cultivars (Figure 1 and Table 4). The number of amplified loci/primer ranged from 8 (ME4/EM8) to 4 (ME3/EM9), with an average of 6 loci per primer (Table 4). In SRAP-PCR reactions, out of the 24 amplified loci, 20 were polymorphic loci with an average mean of 5 polymorphic loci/primer. The percentage of polymorphism ranged from 100% (ME3/EM9, ME4/EM8, and ME5/EM10) to 20% (ME2/EM10), with an average of 80% polymorphism. The reactions of both SRAP and SCoT-PCR-based markers produced 72 loci, 55 of which were polymorphic, 17 were monomorphic, and 6 loci were unique (Table 4).

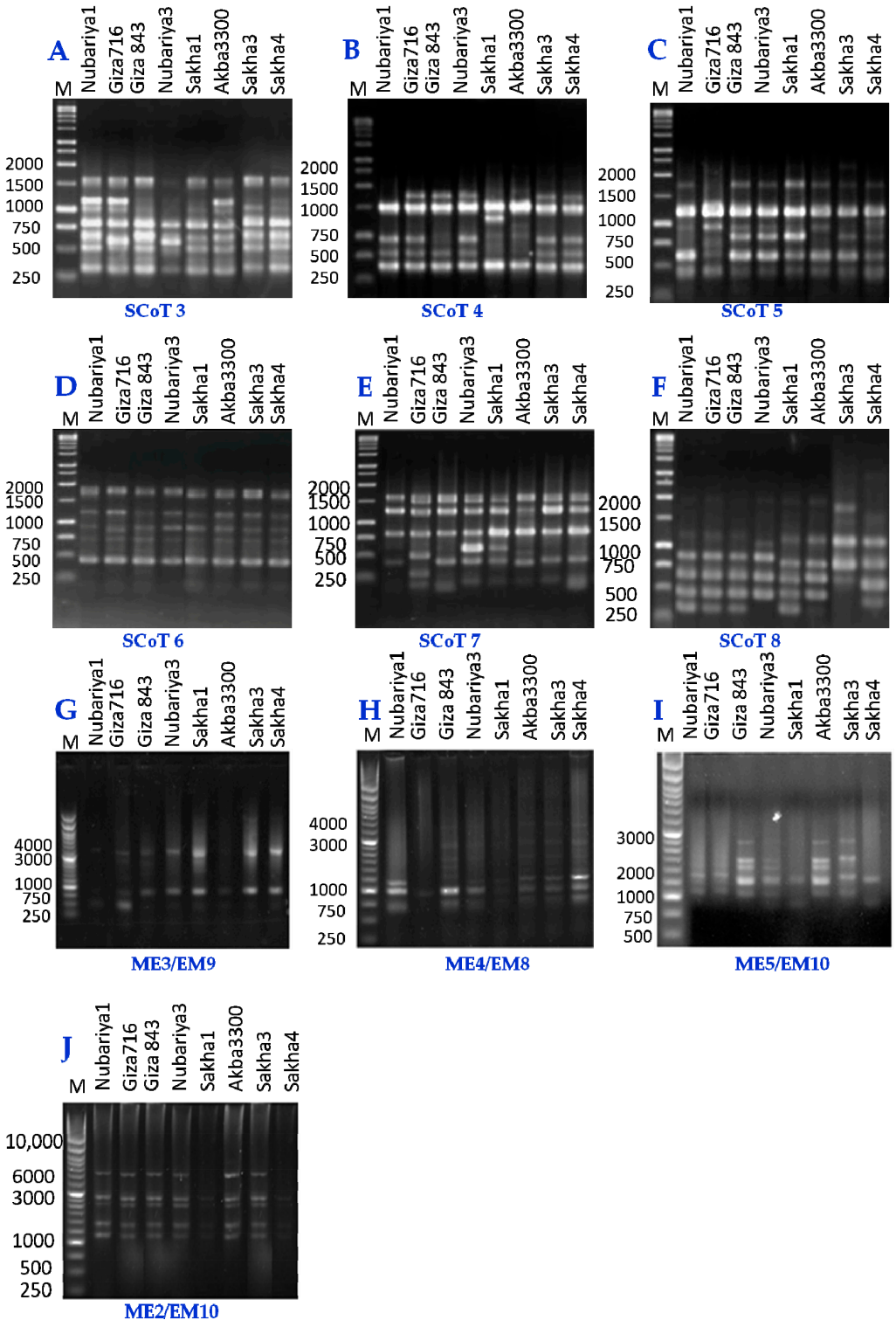

Figure 2. DNA fragment patterns of eight Faba bean cultivars. (**A**–**F**) SCoT-PCR amplification using primers SCoT3, SCoT4, SCoT5, SCoT6, SCoT-7, and SCoT8, respectively. (**G**–**J**) SRAP-PCR amplification using primers ME3/EM9, ME4/EM8, ME5/EM10, and E2/EM10, respectively. M = 1 kbp DNA ladder.

3.3. Genetic Distance and Similarity

The lowest genetic distance (3.16) was presented between Nubariya 1 vs. Nubariya 3, Sakha 3 vs. Sakha 4, and Sakha 4 vs. Akba 3300, while the highest genetic distance (4.90) was between Sakha 4 and Giza 716 (Table 5). The scored data obtained from the ten primers

were analyzed using the Dice coefficient to compute the similarity matrices. As shown in Table 5, the highest similarity was presented between Sakha 4 vs. Akba 3300 (0.894), Sakha 3 vs. Sakha 4 (0.891), and Nubariya 1 vs. Nubariya 3 (0.881), while the lowest similarity (0.732) was between Sakha 3 and Giza 716 (Table 6).

Table 5. Genetic distance among the eight faba bean cultivars based on SCoT and SRAP banding profiles.

	Nubariya 1	Nubariya 3	Giza 843	Giza 716	Sakha 1	Sakha 3	Sakha 4	Akba 3300
Nubariya1	0.00							
Nubariya 3	**3.16**	0.00						
G843	4.24	4.47	0.00					
Giza716	4.47	4.47	4.69	0.00				
Sakha 1	4.24	4.47	4.47	4.69	0.00			
Sakha 3	4.00	4.47	3.74	4.69	4.24	0.00		
Sakha 3	4.47	4.47	3.46	**4.90**	4.24	**3.16**	0.00	
Akba 3300	4.24	4.24	3.74	4.47	3.46	3.46	**3.16**	0.00

Table 6. Similarity coefficient (Dice measurement) of the eight faba bean cultivars based on SCoT and SRAP banding profiles.

	Nubariya 1	Nubariya 3	Giza 843	Giza 716	Sakha 1	Sakha 3	Sakha 4	Akba 3300
Nubariya 1	1							
Nubariya 3	0.881	1						
G843	0.804	0.787	1					
Giza716	0.744	0.750	0.750	1				
Sakha 1	0.780	0.762	0.783	0.718	1			
Sakha 3	0.814	0.773	0.854	**0.732**	0.791	1		
Sakha 4	0.773	0.778	0.878	0.714	0.795	**0.891**	1	
Akba 3300	0.795	0.800	0.857	0.762	0.864	0.870	**0.894**	1

3.4. Phylogeny Analysis

The phylogeny analysis of the combined SCoT and SRAP-PCR banding profiles grouped the eight cultivars into three main clusters. Giza 716 formed an independent cluster (I). The second cluster included Sakha 1, Sakha 3, Sakha 4, and Akba 3300. The third cluster consisted of two cultivars, Nubariya 1 and Nubariya 3 (Figure 3).

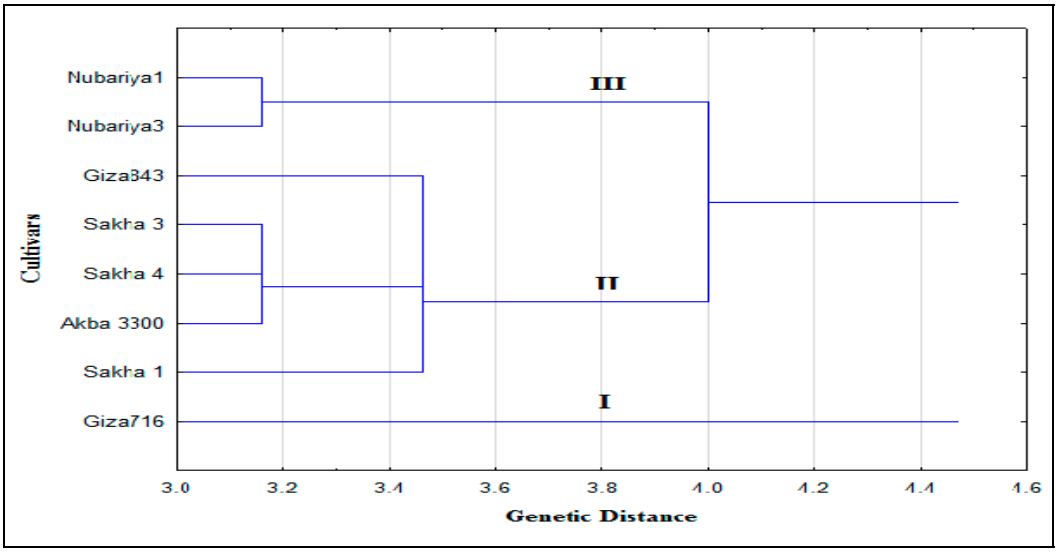

Figure 3. Phylogenetic tree of eight faba bean cultivars revealed based on SCoT and SRAP banding profiles. Note: I means group 1, II means group 2, III means group 3.

4. Discussion

The performance of different faba bean cultivars under drought conditions was assessed in terms of the number of leaves at 30 days. At 60 days, the length of shoots, the length of roots, the dry weight of shoot (g), and the dry weight of root and proline content were recorded. The effect of drought stress shown in Table 2 indicated that the performance of studied cultivars could be significantly affected by drought conditions and normal irrigation regimes. Therefore, these parameters could be used as morphological and physiochemical criteria for detecting drought stress tolerance and susceptibility in faba bean plants. Similar results were obtained by Al-Amri [30], who investigated the variable responses of faba bean plants to drought and waterlogging stresses.

A shortage in water availability results in growth reduction by decreasing shoot and root growth and subsequently reducing the shoot and dry root weights. This reduction may be due to decreased photosynthesis, growth of the plant, expansion, and the division of plant cells [10,11]. Similar results were obtained by Ouzounidou et al. [31], who studied the effect of abiotic stresses on the crop yield of broad bean. These results are in agreement with those obtained by Ouzounidou et al. [31]; Ammar et al. [32] found that drought stress has significant effects on all faba bean traits.

The measurement of proline contents of faba bean leaves cultivars suggested that proline accumulations increased under drought conditions and decreased with the normal water regime. The highest proline content values (4.94, 4.39, and 4.26 mmol/g fresh weight) were measured under 50% field capacity conditions in Nubariya 1, Nubariya 3, and Giza 716, respectively. On the other hand, the cultivars Sakha 1, Sakha 4, Nubariya 1, and Nubariya 3 exhibited the highest proline contents (7.8,7.53, 6.17, and 6.25, respectively) under 25% field capacity treatment. These results indicate that the performance of faba bean cultivars varied with the variation in drought stress levels, and some cultivars such as Sakha 1 and Sakha 4 need higher drought levels to present higher proline content. According to Ammar et al. [32], seedlings of Gazira 2 and Hassawi 2 accumulated the most leaf-free proline under drought conditions. On the other hand, under normal irrigation conditions, the cultivars Gazira 2 and TW showed the lowest proline concentration. These findings revealed that faba bean leaves' proline content increased with drought stress and decreased under normal water conditions. In other plants such as wheat, the proline quantity was increased after drought in [33], pea [34], chickpea [35], sugar beet [36], sesame [37], sunflower [38], upland rice [39], and cotton [40]. According to Ghiabi et al. [41], proline content had an insignificant correlation with regular irrigation and a strong positive correlation with drought tolerance. On the other side, Parchin et al. [42] found an insignificant negative correlation between proline content and drought tolerance. Numerous studies recommended using the accumulation of proline to select genotypes that were tolerant to water stress in rosy periwinkle [43], safflower [44] and sesame [37].

Wide-ranging plant genetic resources that might be used in various breeding programs to produce plants with superior features are a major factor in producing high-yield or tolerant crops [45]. Plant breeders' ability to generate new elite cultivars is considerably increased when they have access to various genetic variations [46]. Breeding strategies that try to select particular traits or natural processes, such as domestication and dispersal, continuously reduce genetic diversity [47]. Nevertheless, new polymorphic bands or alleles can be found and identified through genetic diversity research, enriching any crop's genetic variety [48]. SCoT and SRAP molecular markers were used in this research to evaluate the degree of genetic variation among eight faba bean cultivars. The findings revealed significant genetic variation across faba bean cultivars under study. The high level of polymorphism, 70.93% and 80% scored by SCoT and SRAP, respectively, indicated that the studied faba bean cultivars are highly divergent and suggested that both markers are suitable for studying the genetic variation among closely related cultivars. The SRAP-PCR approach used in the current research is more efficient in determining genetic diversity based on its polymorphic profiles than the SCoT-PCR marker. Similar results were reported by Mahmoud and Abd El-Fatah [49], who used SRAP primers to discover that faba bean

genotypes are highly polymorphic. The assessment of genetic similarity and genetic distance among plant cultivars is helpful in adjusting breeding programs to facilitate the selection of parents. These results suggested that the SCoT and SRAP approaches showed considerable potential for identifying and discriminating faba bean cultivars concerning their tolerance to drought conditions.

Considering the outcomes of the molecular profiles of the faba bean cultivars, a phylogenetic tree was generated to divide the studied cultivars into groups according to the genetic distance scores based on the molecular profiles produced by SCoT and SRAP markers. Important studies were conducted to evaluate the genetic diversity among various species, including phylogeny and alignment [1,2,13,14,50–54]. The current study supports conventional breeding strategies for faba bean genetic development; nevertheless, further approaches such as using chemical and physical mutagens [55], in silico studies [56], genetic engineering [57], and approaches of genome editing [58,59] must be applied in faba bean breeding and improvement for different desirable traits.

5. Conclusions

Significant variations among faba bean cultivars were observed, which will efficiently support the identification of promising parents for breeding abiotic stress-tolerant genotypes. Generally, the morphological, physiochemical and molecular parameters were suitable to assess variations among the faba bean cultivars based on their background regarding drought tolerance. The cultivars Sakha 1, Sakha 4, Nubariya 1, and Nubariya 3 exhibited the best response based on the proline content criterion under the severe drought stress conditions. The combined SCoT- and SRAP-PCR-based markers were significantly helpful for assessing genetic variation in faba bean cultivars. The phylogeny analysis grouped the eight faba bean cultivars into three clusters based on their molecular banding profiles. The cultivars Sakha 1, Sakha 4, Nubariya 1, and Nubariya 3 will be useful parents in the future for breeding drought-resistant varieties in faba bean.

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References

1. Fang, E.F.; Hassanien, A.A.E.; Wong, J.H.; Bah, C.S.F.; Soliman, S.S.; Ng, T.B. Purification and Modes of Antifungal Action by Vicia faba cv. Egypt Trypsin Inhibitor. *J. Agric. Food Chem.* **2010**, *58*, 10729–10735. [CrossRef]
2. Fang, E.F.; Hassanien, A.A.E.; Wong, J.H.; Bah, C.S.F.; Soliman, S.S.; Ng, T.B. Isolation of a new trypsin inhibitor from the Faba bean (*Vicia faba* cv. Giza 843) with potential medicinal applications. *Protein Pept. Lett.* **2011**, *18*, 64–72. [CrossRef]
3. Qahtan, A.A.; Al-Atar, A.; Abdel-Salam, E.M.; El-Sheikh, M.A.; Gaafar, A.-R.Z.; Faisal, M. Genetic diversity and structure analysis of a worldwide collection of faba bean (*Vicia faba*) genotypes using ISSR markers. *Int. J. Agric. Biol.* **2021**, *25*, 683–691. [CrossRef]
4. Maalouf, F.; Hu, J.; O'Sullivan, D.M.; Zong, X.; Hamwieh, A.; Kumar, S.; Baum, M. Breeding and genomics status in faba bean (*Vicia faba*). *Plant Breed.* **2019**, *138*, 465–473. [CrossRef]
5. Alharbi, N.H.; Adhikari, K.N. Factors of yield determination in faba bean (*Vicia faba*). *Crop Pasture Sci.* **2020**, *71*, 305–321. [CrossRef]
6. Banerjee, J.; Das, A.; Parihar, A.; Sharma, R.; Pramanik, K.; Barpete, S. Genomic Designing Towards Development of Abiotic Stress Tolerant Grass Pea for Food and Nutritional Security. In *Genomic Designing for Abiotic Stress Resistant Pulse Crops*; Springer: Cham, Switzerland, 2022; pp. 345–381.
7. Cordea, M.I.; Borsai, O. Salt and Water Stress Responses in Plants. In *Plant Stress Physiology-Perspectives in Agriculture*; IntechOpen: London, UK, 2021.
8. Turner, N.C. Further progress in crop water relations. *Adv. Agron.* **1996**, *58*, 293–338.
9. El-Enany, A.E.; Al-Anazi, A.D.; Dief, N.; Al-Taisan, W.A.A. Role of antioxidant enzymes in amelioration of water deficit and waterlogging stresses on Vigna sinensis plants. *J. Biol. Earth Sci.* **2013**, *3*, B144–B153.
10. Sundaravalli, V.; Paliwal, K.; Ruckmani, A. Effect of water stress on photosynthesis, protein content and nitrate reductase activity of Albizzia seedlings. *J. Plant Biol.-New Delhi* **2005**, *32*, 13.
11. Rodriguez, R.J.; Henson, J.; Van Volkenburgh, E.; Hoy, M.; Wright, L.; Beckwith, F.; Kim, Y.-O.; Redman, R.S. Stress tolerance in plants via habitat-adapted symbiosis. *ISME J.* **2008**, *2*, 404–416. [CrossRef] [PubMed]
12. Varshney, R.K.; Thudi, M.; Roorkiwal, M.; He, W.; Upadhyaya, H.D.; Yang, W.; Bajaj, P.; Cubry, P.; Rathore, A.; Jian, J. Resequencing of 429 chickpea accessions from 45 countries provides insights into genome diversity, domestication and agronomic traits. *Nat. Genet.* **2019**, *51*, 857–864. [CrossRef] [PubMed]
13. Fathy, D.M.; Eldomiaty, A.; El-Fattah, H.I.A.; Mahgoub, E.-S.I.; Hassanin, A.A. Morphological, Biochemical and Molecular Characterization of Rhizobia of Faba Bean Plants Grown in North Nile Delta Egypt. *Pak. J. Biol. Sci.* **2021**, *24*, 672–679. [CrossRef] [PubMed]
14. Al-Khayri, J.M.; Mahdy, E.M.B.; Taha, H.S.A.; Eldomiaty, A.S.; Abd-Elfattah, M.A.; Abdel Latef, A.A.H.; Rezk, A.A.; Shehata, W.F.; Almaghasla, M.I.; Shalaby, T.A.; et al. Genetic and Morphological Diversity Assessment of Five Kalanchoe Genotypes by SCoT, ISSR and RAPD-PCR Markers. *Plants* **2022**, *11*, 1722. [CrossRef] [PubMed]
15. Khaleghi, A.; Naderi, R.; Brunetti, C.; Maserti, B.E.; Salami, S.A.; Babalar, M. Morphological, physiochemical and antioxidant responses of *Maclura pomifera* to drought stress. *Sci. Rep.* **2019**, *9*, 19250. [CrossRef]
16. Hailu, G.; Asfere, Y. The Role of Molecular Markers in Crop Improvement and Plant Breeding Programs: A. *Agric. J.* **2020**, *15*, 171–175.
17. Garcia, A.A.; Benchimol, L.L.; Barbosa, A.M.; Geraldi, I.O.; Souza, C.L., Jr.; de Souza, A.P. Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. *Genet. Mol.* **2004**, *27*, 579–588. [CrossRef]
18. Al-Hadeithi, Z.; Jasim, S.A. Study of Plant Genetic Variation through Molecular Markers: An Overview. *J. Pharm. Res. Int.* **2021**, *33*, 464–473. [CrossRef]
19. Li, G.; Quiros, C.F. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in Brassica. *Theor. Appl. Genet.* **2001**, *103*, 455–461. [CrossRef]
20. Rana, M.; Singh, S.; Bhat, K. Fingerprinting Indian lentil (*Lens culinaris* ssp. In culinaris Medik.) cultivars and landraces for diversity analysis using sequence-related amplified polymorphism (SRAP) markers. In Proceedings of the Fourth International Food and Legumes Research Conference, New Delhi, India, 18–22 October 2009; pp. 617–624.
21. Collard, B.C.; Mackill, D.J. Start codon targeted (SCoT) polymorphism: A simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Mol. Biol. Rep.* **2009**, *27*, 86–93. [CrossRef]
22. Sarkar, D. *Physical and Chemical Methods in Soil Analysis*; New Age International: New York, NY, USA, 2005.
23. Bates, L.S.; Waldren, R.P.; Teare, I. Rapid determination of free proline for water-stress studies. *Plant Soil.* **1973**, *39*, 205–207. [CrossRef]
24. Doyle, J. DNA protocols for plants. In *Molecular Techniques in Taxonomy*; Springer: Berlin/Heidelberg, Germany, 1991; pp. 283–293.
25. Snedecor, G.W.; Cochran, W.G. *Statistical Methods*, 8th ed.; Iowa State University Press: Ames, IA, USA, 1989; p. 1191.
26. Dice, L.R. Measures of the amount of ecologic association between species. *Ecology* **1945**, *26*, 297–302. [CrossRef]
27. Norusis, M. *SPSS For Windows Advanced Statistics Release 6.0*; SPSS Inc.: Chicago, IL, USA, 1993; p. 578.
28. Rokach, L.; Maimon, O. Decision trees. In *Data Mining and Knowledge Discovery Handbook*; Springer: Berlin/Heidelberg, Germany, 2005; pp. 165–192.
29. Weiß, C.H. *Statistica, Version 8*; Statsoft, Inc.: Tulsa, OK, USA, 2007.

30. Al-Amri, S. Differential response of faba bean (*Vicia faba* L.) plants to water deficit and water logging stresses. *Appl. Ecol. Environ. Res.* **2019**, *17*, 6287–6298. [CrossRef]
31. Ouzounidou, G.; Ilias, I.; Giannakoula, A.; Theoharidou, I. Effect of water stress and NaCl triggered changes on yield, physiology, biochemistry of broad bean (*Vicia faba*) plants and on quality of harvested pods. *Biologia* **2014**, *69*, 1010–1017. [CrossRef]
32. Ammar, M.; Anwar, F.; ElHarty, E.; Migdadi, H.; AbdelKhalik, S.; AlFaifi, S.; Farooq, M.; Alghamdi, S. Physiological and yield responses of faba bean (*Vicia faba* L.) to drought stress in managed and open field environments. *J. Agron. Crop Sci.* **2015**, *201*, 280–287. [CrossRef]
33. Johari-Pireivatlou, M. Effect of soil water stress on yield and proline content of four wheat lines. *Afr. J. Biotechnol.* **2010**, *9*, 36–40.
34. Alexieva, V.; Sergiev, I.; Mapelli, S.; Karanov, E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ.* **2001**, *24*, 1337–1344. [CrossRef]
35. Mafakheri, A.; Siosemardeh, A.; Bahramnejad, B.; Struik, P.; Sohrabi, Y. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Aust. J. Crop Sci.* **2010**, *4*, 580–585.
36. Putnik-Delić, M.; Maksimović, I.; Venezia, A.; Nagl, N. Free proline accumulation in young sugar beet plants and in tissue culture explants under water deficiency as tools for assessment of drought tolerance. *Rom. Agric. Res.* **2012**, *30*, 141–148.
37. Kadkhodaie, A.; Razmjoo, J.; Zahedi, M.; Pessarakli, M. Selecting sesame genotypes for drought tolerance based on some physiochemical traits. *J. Agron.* **2014**, *106*, 111–118. [CrossRef]
38. Nazarli, H.; Faraji, F.; Zardashti, M. Effect of drought stress and polymer on osmotic adjustment and photosynthetic pigments of sunflower. *Cercet. Agron. Mold.* **2011**, *1*, 35–41. [CrossRef]
39. Lum, M.; Hanafi, M.; Rafii, Y.; Akmar, A. Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. *Anim. Plant Sci* **2014**, *24*, 1487–1493.
40. Zhang, L.; Peng, J.; Chen, T.; Zhao, X.; Zhang, S.; Liu, S.; Dong, H.; Feng, L.; Yu, S. Effect of drought stress on lipid peroxidation and proline content in cotton roots. *Anim. Plant Sci* **2014**, *24*, 1729–1736.
41. Ghiabi, S.; Sharafi, S.; Talebi, R. Morpho-physiological and biochemical alternation responses in different chickpea (*Cicer arietinum* L.) genotypes under two constructing water regimes. *Int. J. Biosci.* **2013**, *3*, 57–65.
42. Parchin, R.; Najaphy, A.; Mohebodini, M.S.M.; Vaseghi, A.; Sohrabi-Babahadi, F.; Mostafaie, A. Comparing protein pattern and drought tolerant indicators as screening techniques for drought tolerance in common wheat genotypes. *Int. J. Plant Anim. Environ. Sci.* **2014**, *4*, 251–258.
43. Jaleel, C.A.; Gopi, R.; Sankar, B.; Manivannan, P.; Kishorekumar, A.; Sridharan, R.; Panneerselvam, R. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in Catharanthus roseus seedlings under salt stress. *S. Afr. J. Bot.* **2007**, *73*, 190–195. [CrossRef]
44. Amini, H.; Arzani, A.; Karami, M. Effect of water deficiency on seed quality and physiological traits of different safflower genotypes. *Turk. J. Biol.* **2014**, *38*, 271–282. [CrossRef]
45. Yadav, S.; Verma, N.; Singh, A.; Singh, N.; Rana, S.; Ranga, S.; Kumar, K. Diversity and development in fababean. *Legume Res. Int. J.* **2017**, *40*, 618–623.
46. Galluzzi, G.; Seyoum, A.; Halewood, M.; López Noriega, I.; Welch, E.W. The role of genetic resources in breeding for climate change: The case of public breeding programmes in eighteen developing countries. *Plants* **2020**, *9*, 1129. [CrossRef]
47. Louwaars, N.P. Plant breeding and diversity: A troubled relationship? *Euphytica* **2018**, *214*, 1–9. [CrossRef]
48. Alghamdi, S.S.; Migdadi, H.M.; Khan, M.A.; Afzal, M. AFLP-Based Analysis of Variation and Population Structure in Mutagenesis Induced Faba Bean. *Diversity* **2020**, *12*, 303.
49. Mahmoud, A.F.; Abd El-Fatah, B.E. Genetic diversity studies and identification of molecular and biochemical markers associated with fusarium wilt resistance in cultivated faba bean (*Vicia faba*). *Plant Pathol. J.* **2020**, *36*, 11. [CrossRef]
50. Zong, X.; Yang, T.; Liu, R. Faba Bean (*Vicia faba* L.) Breeding. In *Advances in Plant Breeding Strategies: Legumes*; Springer: Berlin/Heidelberg, Germany, 2019.
51. Raza, S.H.A.; Hassanin, A.A.; Dhshan, A.I.; Abdelnour, S.A.; Khan, R.; Mei, C.; Zan, L. In silico genomic and proteomic analyses of three heat shock proteins (HSP70, HSP90-α, and HSP90-β) in even-toed ungulates. *Electron. J. Biotechnol.* **2021**, *53*, 61–70. [CrossRef]
52. Heakel, R.M. Analysis of genetic diversity among a population of canola genotypes as reveled by ISSR-PCR and their associations to seed yield and oil content. *Ann. Agric. Sci. Moshtohor.* **2019**, *57*, 425–434. [CrossRef]
53. Achimón, F.; Johnson, L.A.; Cocucci, A.A.; Sérsic, A.N.; Baranzelli, M.C. Species tree phylogeny, character evolution, and biogeography of the Patagonian genus Anarthrophyllum Benth.(Fabaceae). *Org. Divers. Evol.* **2018**, *18*, 71–86. [CrossRef]
54. Hassanin, A.A.; Osman, A.; Atallah, O.O.; El-Saadony, M.T.; Abdelnour, S.A.; Taha, H.S.; Awad, M.F.; Elkashef, H.; Ahmed, A.E.; Abd El-Rahim, I. Phylogenetic comparative analysis: Chemical and biological features of caseins (alpha-S-1, alpha-S-2, beta-and kappa-) in domestic dairy animals. *Front. Vet. Sci.* **2022**, *9*, 952319. [CrossRef] [PubMed]
55. Ghareeb, Y.E.; Soliman, S.S.; Ismail, T.A.; Hassan, M.A.; Abdelkader, M.A.; Abdel Latef, A.A.H.; Al-Khayri, J.M.; ALshamrani, S.M.; Safhi, F.A.; Awad, M.F.; et al. Improvement of German Chamomile (*Matricaria recutita* L.) for Mechanical Harvesting, High Flower Yield and Essential Oil Content Using Physical and Chemical Mutagenesis. *Plants* **2022**, *11*, 2940. [CrossRef] [PubMed]
56. Al-Khayri, J.M.; Abdel Latef, A.A.H.; Taha, H.S.A.; Eldomiaty, A.S.; Abd-Elfattah, M.A.; Rezk, A.A.; Shehata, W.F.; Awad, M.; Shalaby, T.A.; Sattar, M.N.; et al. In silico profiling of proline biosynthesis and degradation related genes during fruit development of tomato. *SABRAO J. Breed. Genet.* **2022**, *54*, 549–564. [CrossRef]

57. Hassanin, A.A.; Soliman, S.; Ismail, T.; Amin, M. The role of SLMYB gene in tomato fruit development. *Zagazig J. Agric. Res.* **2017**, *44*, 969–988.
58. Abdelnour, S.A.; Xie, L.; Hassanin, A.A.; Zuo, E.; Lu, Y. The Potential of CRISPR/Cas9 Gene Editing as a Treatment Strategy for Inherited Diseases. *Front. Cell Dev. Biol.* **2021**, *9*, 699597. [CrossRef]
59. Raza, S.H.A.; Hassanin, A.A.; Pant, S.D.; Bing, S.; Sitohy, M.Z.; Abdelnour, S.A.; Alotaibi, M.A.; Al-Hazani, T.M.; Abd El-Aziz, A.H.; Cheng, G. Potentials, prospects and applications of genome editing technologies in livestock production. *Saudi J. Biol. Sci.* **2021**, *29*, 1928–1935. [CrossRef]