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Temperature Effects on Seed Germination and Seedling Biochemical Profile of Cannabis Landraces

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Abstract: This study investigated the effect of temperature on the germination and seedling biochemical profiles of eight cannabis landraces, namely Ladysmith Ugwayi wesiZulu (L1) and Iswazi (L2), Durban Poison (H1), Bergville Ugwayi wesiZulu (B1), Natal (B2), and Iswazi (B3), and Msinga Ugwayi wesiZulu (M1) and Iswazi (M2). Seed viability, germination rate, and germination percentage were evaluated along with seedling amino acids, carbohydrates, and fatty acids methyl esters (FAMEs) under day/night temperature regimes of 20/15 °C, 30/25 °C, and 40/35 °C. Results showed a significant effect ($p < 0.001$) of temperature on germination percentage, rate, and biochemical profiles of cannabis landraces. Landraces L1, B1, H1, B2, and M1 had higher germination at 20/15 °C, while B3, M2, and L2 performed better at 30/25 °C. Biochemical profiles varied with temperature and landraces. Amino acid content increased with temperature but did not correlate with germination indexes. Carbohydrates and FAMEs decreased with rising temperature, peaking at 30/25 °C. FAMEs strongly correlated with germination indexes, linking lipid composition to seed performance. Sorbitol positively correlated with germination, while glucose and fructose showed indirect correlations. This study underscores the impact of temperature on germination and the biochemical profiles of cannabis landraces, highlighting the importance of considering genotype-specific responses in varietal selection.

Keywords: physiology; seed germination index; amino acids; membrane fluidity; fatty acids; carbohydrates

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1. Introduction

Temperature is one of the most important environmental factors regulating seed germination by influencing imbibition and the mobilization of storage reserves and, ultimately, modifying germination percentage and rate [1–7]. During imbibition, the appropriate restructuring of membranes is influenced by temperature by altering their permeability and fluidity characteristics, facilitating or limiting cellular leakage [8]. On the other hand, temperature influences the supply of substrates necessary for seed reserve mobilization, including water, and consequently affects plant growth and development [2,9].

Generally, carbohydrates, such as sucrose, glucose, and fructose, are mobilized during germination from seed storage tissues to organs, such as the stem and radicle, where they support growth and maintain the osmotic homeostasis of cells [10]. These carbohydrates decrease with higher temperatures but accumulate in response to low-temperature stress [11,12]. Amino acid levels tend to increase with rising temperatures due to their role in stabilizing membranes and regulating cellular osmotic pressure [11,13,14]. On the other hand, plant lipid composition adjusts to maintain membrane fluidity, making membranes more rigid at low temperatures to enhance seed performance, while higher temperatures

increase membrane fluidity [15–17]. Thus, the mobilization of seed reserves and the seed germination process differ under various temperature conditions, making temperature a crucial factor influencing germination [2,9,10,16].

Temperature also plays a significant role in determining the consistency of seed germination and distribution and adaptation of species beyond their area of origin [5,18]. The effect of temperature on germination usually varies with species and genotype [3,19]. However, the optimum germination temperature promotes the highest germination percentage and rate within the shortest period [3,19]. On the other hand, temperatures outside the normal limits, such as chilling and high temperatures, may cause secondary dormancy in the seed when conditions are not favorable for seedling establishment, ultimately negatively affecting the germination percentage and germination rate index [1,6,20].

Cannabis sativa L., commonly known as marijuana, dagga, or hemp, is grown worldwide for its oily seeds and long, durable fiber [21–23]. Marijuana or dagga is also grown for its drug effects caused by a compound called (-)-trans-Δ9-tetrahydrocannabinol (Δ9-THC), which led to this crop being banned for many centuries [21,24]. The seeds are known to germinate well under temperatures ranging from 10 to 30 °C [25–28]. However, the optimal germination temperature varies with genotype and geographical location [29]. For instance, 'Georgina', 'Victoria', and Northern European hemp varieties thrive in temperatures up to 30 °C, while the germination of 'Bama' fire hemp and Canadian cultivars declined at temperatures above 25 °C [25,27,29].

Although previous studies have emphasized the influence of temperature on physiological aspects during cannabis germination, there is very little information on the effect of alternating temperature regimes on the biochemical processes [1,3,5,6]. Furthermore, cannabis seeds are known for being rich in polyunsaturated fatty acids (PUFA), specifically linoleic acid (LA, omega-6) and α-linolenic acid (ALA, omega-3) [30,31]. Therefore, it is critical to evaluate the variations in the composition of these fatty acids and other biochemicals during seed germination in response to different temperature regimes.

Additionally, there are limited studies on the effect of temperature on the germination of South African genotypes due to the previous legal restriction attributed to the intoxicating effects of Δ^9-THC. However, public perception and laws in many countries, such as the United States, Canada, South Africa, and Germany, have recently begun acknowledging cannabis as a plant of both medical and agricultural value [21,24,32–34]. Therefore, information, such as optimal germination temperature, as well as the physiological and biochemical responses of the cannabis towards the germination temperature, is vital for variety selection and improvement, planting date, and management practices for specific locations and seed lots [35]. Therefore, the current study aimed to investigate the effect of different temperature regimes on the physiological factors of various landraces during seed germination and the biochemical factors of the seedlings.

2. Materials and Methods

2.1. Landrace Collection

Seeds of eight cannabis landraces were collected from local growers in four regions of KwaZulu-Natal Province in South Africa, namely, Bergville, Ladysmith, Hammersdale, and Msinga. The collected cannabis landraces were assigned codes to ensure uniform naming of landraces for this experiment, as detailed in Table 1.

The collected seed samples were stored for 5 days at room temperature in the Horticultural Sciences Laboratory of the University of KwaZulu-Natal, Pietermaritzburg in South Africa (latitude: −29.636311 and longitude: 30.409060), for further analysis. Prior to germination studies, the cannabis seeds were sorted based on color. Dark-colored seeds were associated with maturity; whereas, greenish-colored seeds were associated with immaturity [36]. Therefore, dark-colored seeds were used for the experiment. Seeds showing defects were removed from the seed lot.

Table 1. Geographical information on the source of landraces and the codes assigned to them.

Location	Geographic Information					Landrace Name	Code
	Latitude	Longitude	Elevation	Average Rainfall	Average Temperature		
Ladysmith	−28.57533	29.85948	1009 m	108.69 mm	23.31 °C	Ugwayi wesiZulu	L1
						Iswazi	L2
Bergville	−28.66768	29.03411	1137 m	109.36 mm	23.45 °C	Natal	B2
	−28.67754	29.12555				Ugwayi wesiZulu	B1
						Iswazi	B3
Hammersdale	−29.8709	30.63352	597 m	110.34 mm	23.66 °C	Durban Poison	H1
Msinga	−28.67989	30.27473	559 m	340.0 mm	23.42 °C	Ugwayi wesiZulu	M1
						Iswazi	M2

2.2. Experimental Design

The experiment was structured using a factorial completely randomized design consisting of 8 landraces and 3 temperature levels. Seeds were subjected to different day/night alternating temperature levels of 20/15 °C, 30/25 °C, and 40/35 °C.

2.3. Seed Viability Test

A seed viability test was conducted before seed germination using 2,3,5-triphenyltetrazolium chloride (TCC) (Sigma Aldrich, Merck KGaA, 64271 Darmstadt, Germany), following the procedures by [37]. Seed samples were preconditioned by soaking in distilled water for 18 h in an incubator set at 25 °C and then excised with a scalpel blade through the embryo. The excised seeds were immersed in a 1% TCC for 12 h at room temperature. The experiment was replicated three times, and each replicate consisted of 25 seeds. Seed viability evaluation was carried out by placing the seeds in the categories viable and non-viable, according to the coloration of the embryonic axis. Viable seeds were characterized by embryos showing a uniform shiny pink color, and non-viable seeds were characterized by embryos showing a totally white color [38]. Only the percentage of viable seeds was recorded.

2.4. Germination Test

Seeds were soaked in distilled water for 24 h to promote germination. Thereafter, seeds were air-dried and placed in 9 cm sterile Petri dishes containing cotton pads soaked with distilled water. Each Petri dish had 25 seeds, and each experiment was replicated three times for the different alternating temperatures of 20/15, 30/25, and 40/35 °C. The seeded Petri dishes were then transferred into a germination chamber (Micro-Clima Arabidopsis Chamber, ECD01E, Snijders, The Netherlands) maintained at different alternating temperatures of 20/15, 30/25, and 40/35 °C and a light/night cycle of 16/8 h. The germination progress was monitored daily, and the number of germinated seeds was recorded for each replicate for 10 days. A seed was considered to have germinated when the emerging radical elongated to a length of 2 mm or more. The germination percentage (GP) was calculated according to [39] using Equation (1).

$$\mathrm{GP}(\%) = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100 \quad (1)$$

The germination rate index (GRI) was calculated according to [40] using Equation (2) and was expressed as a percentage per day (%/day).

$$\mathrm{GRI}(\%/\text{day}) = \frac{\text{Percentage of germinated seeds}}{\text{Day of first count}} + \ldots + \frac{\text{Percentage of germinated seeds}}{\text{Day of final count}} \quad (2)$$

2.5. Biochemical Profiling

Four landraces were randomly selected for metabolic profiling of seedlings. These included B1 for 'Ugwayi wesiZulu', B2 for 'Natal', H1 for 'Durban Poison', and L2 for 'Iswazi'. The seedlings were individually frozen at −70 °C for 24 h, and then, they were freeze-dried for 4 days at −126.5 °C in a Larry Virtis 255L (SP Scientific, Warminster, PA, USA). The dried samples were ground into a fine powder using a mortar and pestle and stored in a chest freezer (Defy Appliances, Durban, KZN, South Africa) at −5 °C until further analysis.

2.5.1. Extraction and Quantification of Carbohydrates

The carbohydrates were extracted and quantified using a method previously described by [41] with some modifications. An amount of 50 mg of powdered samples from each of the 3 replicates per landrace was mixed with 1 mL of 70% (*v*/*v*) methanol (MeOH) (Minema Chemicals (Pty) Ltd., 34 Ridge Rd, Laser Park; Johannesburg, South Africa). The samples were extracted at 60 °C for 3 h. An extract (130 µL) was transferred into a 2 mL polypropylene tube and dried at 35 °C overnight in a speed vac (Model number: 7810010, Labconco, Kansas City, MO, USA) at 825 rpm. The dried samples were derivatized by adding 100 µL of methoxyamine (Sigma-Aldrich, St. Louis, MO, USA) at 50 °C for 2 h, followed by the addition of 30 µL of N,O-Bis-(trimethylsilyl)trifluoroacetamide (BSTFA) (Sigma-Aldrich, St. Louis, MO, USA) and further derivatized at 70 °C for 30 min. The samples were transferred into an insert, positioned in a 2 mL gas chromatograph (GC) vial, and 1 µL was injected into the gas chromatography mass spectrometry system (GC-MS) (6890N, Agilent technologies network and 5975, Agilent Technologies Inc., Palo Alto, CA, USA) in splitless mode.

Separation was performed on a gas chromatograph (6890N, Agilent technologies network) coupled to Agilent technology inert XL EI/CI Mass Selective Detector (MSD) (5975, Agilent Technologies Inc., Palo Alto, CA, USA). The GC-MS system was coupled to a CTC Analytics PAL autosampler. The carbohydrates were separated on a non-polar ZB-5MS (30 m, 0.25 mm ID, 0.25 µm film thickness) capillary column (Phenomenex, Torrance, CA, USA). Helium was used as the carrier gas at a 1 mL/min flow rate. The injector temperature was maintained at 250 °C. The oven temperature was programmed at 80 °C for 1 min, ramped up to 300 °C at a rate of 7 °C/min, and then held for 2 min. The detection process was performed in the full scanning mode from 50–550 (*m*/*z*). The chromatograms were analyzed by checking the MS database and GC/MS database of NIST 14. lib. The analytical performances were evaluated by calculating the relative standard deviation (RSD) of peak area and count of metabolites. The final results were expressed in milligrams per gram sample dry mass (DM).

2.5.2. Extraction and Quantification of Amino Acids

The amino acids were extracted and quantified using a method previously described by [42,43], with some modifications. A 4 mL sample of 6 M hydrochloric acid (HCl) (Minema Chemicals (Pty) Ltd., 34 Ridge Rd, Laser Park; Johannesburg, South Africa) was added to 100 mg of freeze-dried and powdered samples. The samples were hydrolyzed for 24 h at 110 °C, cooled to room temperature, and diluted at 1:9 with 70% methanol (*v*/*v*). An amount of 130 µL was transferred into a 2 mL polypropylene tube and dried completely under a gentle stream of nitrogen. The samples were reconstituted and derivatized with 30 µL N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) (Sigma-Aldrich, St. Louis, MO, USA) and 100 µL acetonitrile at 100 °C for 1 h. After cooling at room temperature, the samples were injected into the GC-MS instrument.

Separation was performed on a gas chromatograph (6890N, Agilent technologies network) coupled to Agilent technology inert XL EI/CI Mass Selective Detector (MSD) (5975, Agilent Technologies Inc., Palo Alto, CA, USA). The GC-MS system was coupled to a CTC Analytics PAL autosampler. Separation of amino acids was performed on a ZB-5MS (30 m, 0.25 mm ID, 0.25 µm film thickness) capillary column. Helium was used

as the carrier gas at a 1 mL/min flow rate. The injector temperature was maintained at 250 °C. A 1 µL of the sample was injected in splitless mode. The oven temperature was programmed at 100 °C for 5 min and ramped up to 325 °C at a rate of 20 °C/min for 4 min and, thereafter, held for 15 s. The MSD was operated in scan/sim mode, and the source and quad temperatures were maintained at 240 °C and 150 °C, respectively. The transfer line temperature was maintained at 250 °C. The mass spectrometer was operated under electron impact mode at an ionization energy of 70 eV, scanning from 40 to 650 *m/z*. Free amino acids were identified both by their retention time and by comparison of their characteristic *m/z* ions with those published by [43]. The quantification was carried out in the selected ion monitoring (SIM) mode. The final results were expressed in milligrams per gram sample dry mass.

2.5.3. Extraction and Quantification of Fatty Acid Methyl Esters (FAMEs)

The extraction and quantification of FAMEs were carried out using a method previously described by [44] with some modifications. Briefly, 2 mL of 2:1 chloroform (Minema Chemicals (Pty) Ltd., 34 Ridge Rd, Laser Park; Johannesburg, South Africa): methanol (*v*/*v*) was added to 100 mg of freeze-dried and powdered samples. The samples were vortexed using a Fisherbrand™ ZX3 vortex mixer (Fisher Scientific, Bishop Meadow Road, Loughborough, Leicestershire, UK) and sonicated using a Fisherbrand™ Q705 Sonicator with Probe (Fisher Scientific, Bishop Meadow Road, Loughborough, Leicestershire, UK) at room temperature for 30 min, after which the samples were centrifuged at 3000 rpm for 1 min. The bottom layer (chloroform), measuring 200 µL, was then dried completely using a gentle stream of nitrogen. Subsequently, it was reconstituted and vortexed with 170 µL of methyl tert-butyl ether (MTBE) (Sigma-Aldrich, St. Louis, MO, USA) and 30 µL of trimethylsulfonium hydroxide (TMSH) (Sigma-Aldrich, St. Louis, MO, USA). An amount of 1 µL of the derivatized samples was injected in a 5:1 split ratio into the GC-FID.

Separation was performed on a gas chromatograph (6890N, Agilent Technologies) coupled to a flame ionization detector (FID). The FAMEs were separated on a polar RT-2560 (100 m, 0.25 mm ID, 0.20 µm film thickness) (Restek, Bellefonte, PA, USA) capillary column. Hydrogen was used as the carrier gas at a 1.2 mL/min flow rate. The injector temperature was maintained at 240 °C. Exactly 1 µL of the sample was injected in a 5:1 split ratio. The oven temperature was programmed as follows: 100 °C for 4 min, ramped to 240 °C at a rate of 3 °C/min for 10 min. Compounds were identified by comparing retention times and mass fragmentation patterns with standards and the NIST library. Peaks were matched with known FAME standards. Quantitation of fatty acids used response factors from TG standards in mixtures, with TG-9:0 as the internal standard (ISTD). The final results were expressed in milligrams per gram sample dry mass.

2.6. Data Analysis

The data were analyzed using GenStat®, 20.1 Edition (VSN International, Hamel Hampstead, UK, 2020) at the 5% significance level. The means of significantly different variables were separated using Tukey's test. Additionally, the statistical data analysis for multivariate analysis, such as correlation and principal component analysis (PCA), was conducted using Statistical Computing Environment, R software, version 4.4.0 using the RStudio platform (Posit Software, PBC, 250 Northern Ave Suite 420 Boston, MA 02210, USA). The corrplot package was used for correlation; whereas, the FactoMineR package (version 2.11) was used for PCA.

3. Results

3.1. Seed Viability

There were no significant differences between landraces in relation to seed viability ($p = 0.057$). For instance, B1, B2, L2, and M1 were significantly different from M2. Amongst the landraces, B1 and M2 had the highest and lowest seed viability at 82.00 and 97.67%, respectively (Figure 1).

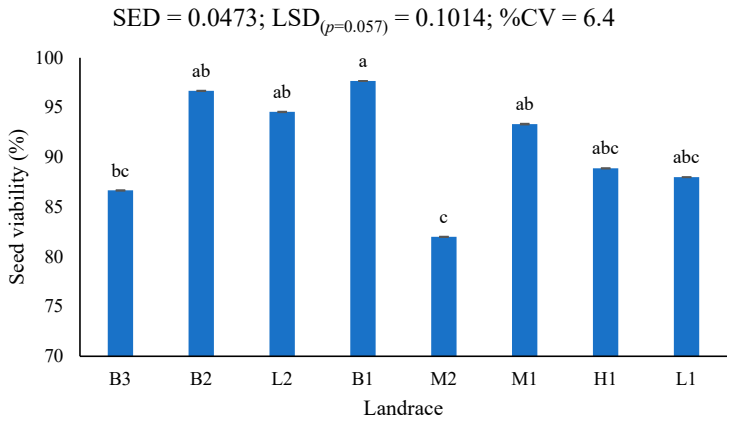

Figure 1. Seed viability of eight cannabis landraces before they were subjected to different temperature regimes ($LSD_{(p=0.05)}$ = 0.1014; number of replicates (n) = 3). Columns with the same letters are not significantly different, while columns with different letters are significantly different, according to Tukey's test ($p < 0.05$). SED = standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation. L1 and L2 = Ladysmith Ugwayi wesiZulu and Iswazi, respectively; H1 = Durban Poison; B1, B2, and B3 = Bergville Ugwayi wesiZulu, Natal and Iswazi, respectively; M1 and M2 = Msinga Ugwayi wesiZulu and Iswazi, respectively.

3.2. Germination

3.2.1. Germination Percentage

The landraces, temperature regimes, and their interactions showed significant differences ($p < 0.001$) in germination percentage. The results revealed that B3, L2, and M2 had the highest germination percentages of 82.78, 89.44, and 77.78% under 30/25 °C (Figure 2). Under 20/15 °C, the landraces B2, B1, M1, and L1, respectively, exhibited the germination percentage of 93.93, 86.67, 80.56, and 60.00%. H1 had the highest germination of 63.89% under 20/15 °C, followed by 62.78% under 40/35 °C. Notably, the germination percentages for B2 decreased with the increasing temperature.

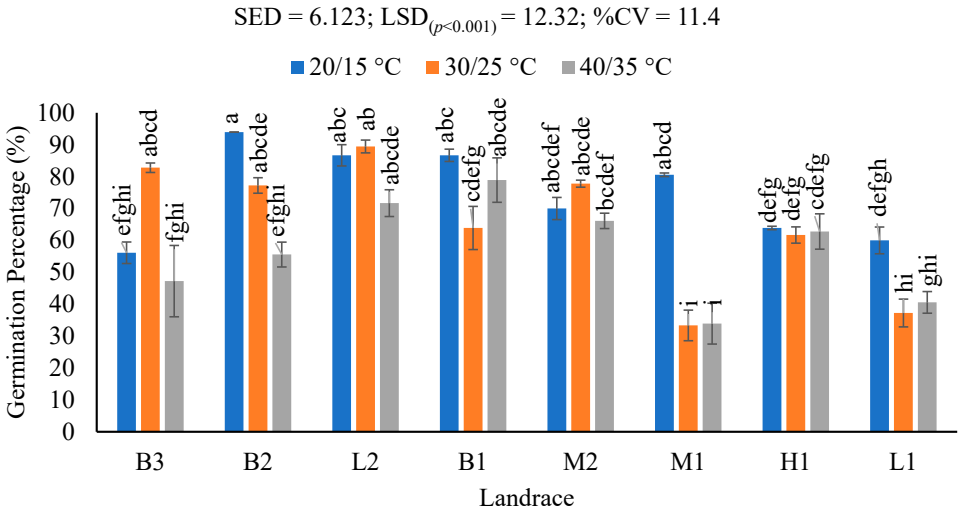

Figure 2. The germination percentage for the interaction effect between three different temperature levels and eight cannabis landraces ($LSD_{(p < 0.001)}$ = 12.32; n = 3). LSD 5% = least significance difference at 5 percent. Columns with the same letters are not significantly different, while columns with different letters are significantly different, according to Tukey's test ($p < 0.05$). SED = standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation. L1 and L2 = Ladysmith Ugwayi wesiZulu and Iswazi, respectively; H1 = Durban Poison; B1, B2, and B3 = Bergville Ugwayi wesiZulu, Natal and Iswazi, respectively; M1 and M2 = Msinga Ugwayi wesiZulu and Iswazi, respectively.

3.2.2. Germination Rate Index

The landraces, temperature levels, and their interactions showed significant differences ($p < 0.001$) in germination rate index. The germination rate index decreased with the increasing temperature in landrace M1, as it had the lowest at 20.98%/day under 20/15 °C compared with other temperature regimes (Figure 3). Under 30/25 °C, B2, H1, M2, B3, L1, and L2 had the highest germination rate indexes of 44.70, 34.48, 41.05, 32.92, 14.77, and 57.12%/day, respectively, compared with other temperature regimes. Landraces B2 and L2 had the lowest germination rate indexes under 40/35 °C at 27.43 and 28.08%/day, respectively. B3, H1, M2, and L1 had the lowest germination rate indexes under 20/15 °C at 12.91, 24.36, 22.04, and 12.25%/day, respectively. On the other hand, the germination rate index for landrace B1 increased with the increasing temperature, exhibiting a germination rate index of 47.95%/day under 40/35 °C compared with 34.38 and 36.50%/day recorded under 20/15 and 30/25 °C, respectively.

$$SED = 3.404; LSD_{(p<0.001)} = 6.851; CV\% = 15.0$$

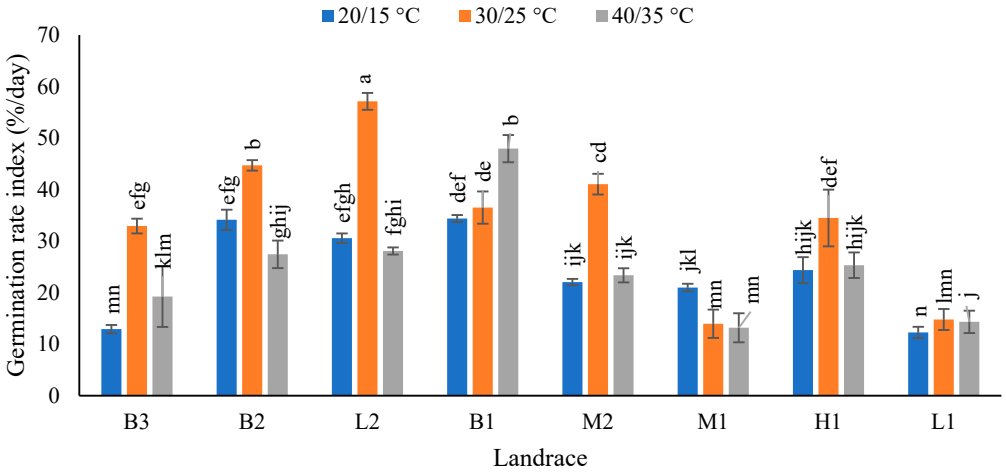

Figure 3. Germination rate index for the interaction effect between three different alternating temperature levels and eight cannabis landraces ($LSD_{(p<0.05)} = 6.851$; n = 3). Columns with the same letters are not significantly different, while columns with different letters are significantly different, according to Tukey's test ($p < 0.05$). SED = standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation. L1 and L2 = Ladysmith Ugwayi wesiZulu and Iswazi, respectively; H1 = Durban Poison; B1, B2, and B3 = Bergville Ugwayi wesiZulu, Natal and Iswazi, respectively; M1 and M2 = Msinga Ugwayi wesiZulu and Iswazi, respectively.

3.3. Biochemical Profiling

3.3.1. Carbohydrate Concentration and Composition

The landraces, temperature levels, and their interactions showed significant differences ($p < 0.001$) in the sugar composition of the cannabis seedlings. The concentration of glucose, fructose, sucrose, mannitol, myo-inositol, and sorbitol varied with landrace and temperature levels. For instance, glucose concentration was generally higher compared with the other sugars in the seeds of cannabis landraces at all temperature levels, followed by fructose concentration (Table 2). Conversely, the concentration of mannitol was generally lowest under the 20/15 °C regime; whereas, the concentration of sucrose was generally lowest under the 30/25 and 40/35 °C regimes. B2 exhibited the highest glucose concentration of 30.21 mg/g DM under 20/15 °C and 31.06 mg/g DM under 30/25 °C. Similarly, B2 had the highest mannitol concentration of 6.57 mg/g DM under 40/35 °C. In contrast, B2 exhibited the lowest mannitol concentration of 1.61 mg/g DM under 20/15 °C and the lowest sucrose concentrations of 2.21 and 0.73 mg/g DM under 30/25 and 40/35 °C, respectively.

Table 2. The concentration of sugars (sucrose, glucose, sorbitol, myo-inositol, mannitol, and fructose) under three different temperature levels and four cannabis landraces (number of replicates (n) = 3). Means with different letters are significantly different, according to Tukey's test ($p < 0.05$). SED = Standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation; DM = dry mass. L2 = Ladysmith Iswazi; H1 = Durban Poison; B1 and B2 = Bergville Ugwayi wesiZulu and Natal, respectively.

Landrace	Temperature (°C)	Sucrose (mg/g DM)	Glucose (mg/g DM)	Sorbitol (mg/g DM)	Myo-Inositol (mg/g DM)	Mannitol (mg/g DM)	Fructose (mg/g DM)
B2	20/15	3.31 a	30.21 bc	8.03 a	2.98 d	1.61 g	14.19 d
	30/25	2.21 bc	31.06 b	5.21 b	4.78 c	2.34 f	19.69 b
	40/35	0.73 f	5.94 g	2.21 de	1.97 ef	6.57 b	4.00 f
H1	20/15	2.62 ab	5.14 g	1.71 ef	1.62 f	1.09 h	2.79 f
	30/25	1.74 cd	26.12 d	3.60 c	3.17 d	2.97 e	20.60 b
	40/35	1.18 def	29.45 c	3.99 c	6.45 b	6.29 c	21.36 b
B1	20/15	1.50 cdef	33.59 a	4.26 c	7.68 a	7.16 a	26.45 a
	30/25	2.12 bc	23.25 e	1.59 ef	4.71 c	2.59 f	16.88 c
	40/35	0.84 ef	6.42 g	2.76 d	1.98 ef	5.49 d	4.27 f
L2	20/15	1.57 cde	33.17 a	3.89 c	2.06 ef	1.05 h	13.67 d
	30/25	1.52 cdef	15.49 f	2.56 d	2.61 de	0.99 h	10.48 e
	40/35	0.73 f	5.75 g	1.44 f	1.71 f	5.54 d	4.28 f
p-value	Landrace	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Treatment	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Landrace × Temperature	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	SED	0.223	0.389	0.184	0.219	0.072	0.612
	LSD	0.462	0.807	0.381	0.453	0.150	1.270
	%CV	16.3	2.3	6.5	7.7	2.4	5.7

The landraces H1, L2, and B1 had the highest glucose concentrations of 5.14, 33.17, and 33.59 mg/g DM under 20/15 °C, 26.12, 15.49, and 23.25 mg/g DM under 30/25 °C, and 29.45, 5.75, and 6.42 mg/g DM under 40/35 °C, respectively. On the other hand, H1 had the lowest mannitol concentration at 1.08 mg/g DM under 20/15 °C and the lowest sucrose concentrations at 1.74 and 1.18 mg/g DM under 30/25 and 40/35 °C, respectively. The concentration of sucrose was lowest in the 'B1' landrace, where 1.50 and 0.84 mg/g DM were observed under 20/15 and 40/35 °C, respectively. Additionally, B1 showed the lowest sorbitol concentration of 1.59 mg/g DM under 30/25 °C. L2 had the lowest mannitol concentrations of 1.05 and 0.99 mg/g DM under 20/15 and 30/25 °C, respectively. Under 40/35 °C, L2 had the lowest sucrose concentration of 0.73 mg/g DM.

3.3.2. Amino Acids Concentration and Composition

The landraces, temperature levels, and their interactions showed significant differences ($p < 0.001$) in the amino acids of the cannabis seedlings. Seventeen amino acids were identified in the cannabis landrace seedlings grown at varying temperatures (Table 3). Notably, the concentration of amino acids increased with the increasing temperature in H1, B1, and L2. In B2, the concentration of amino acids was the highest under 20/15 °C, reduced to the lowest under 30/25 °C, and then increased at 40/35 °C. Amongst the amino acids, cysteine concentration was the lowest in all landraces and under all temperature levels; whereas, lysine, leucine, and valine concentrations were generally higher. The leucine concentration in B1 increased from 10.78 to 11.97 and 17.11 mg/g DM under 20/15 °C, 30/25 °C, and 40/35 °C, with the corresponding valine concentration having increased from 7.92 to 9.79 and 16.01 mg/g DM, respectively. Additionally, cysteine concentration increased with the increasing temperature, except in B2, as it recorded 6.25 mg/g DM under 20/15 °C, which was higher compared with 0.05 and 0.50 mg/g DM recorded under 30/25 and 40/35 °C, respectively.

Table 3. The concentration of 17 different amino acids under three different temperature levels and four cannabis landraces (n = 3). Means with different letters are significantly different, according to Tukey's test ($p < 0.05$). SED = Standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation; DM = dry mass. L2 = Ladysmith Iswazi; H1 = Durban Poison; B1 and B2 = Bergville Ugwayi wesiZulu and Natal, respectively.

Landrace	Temperature (°C)	Amino Acids (mg/g DW)																
		Alanine	Aspartic	Cysteine	Cystine	Glutamic Acid	Glycine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Proline	Serine	Threonine	Tyrosine	Valine
'B2'	20/15	11.96 a	12.76 b	6.26 a	11.25 a	12.82 a	12.88 a	17.52 a	15.31 a	18.37 a	28.00 a	7.83 a	14.80 a	15.59 a	7.81 ab	12.88 a	10.41 a	16.99 a
	30/25	4.11 i	2.85 h	0.05 d	2.19 f	2.56 h	3.87 g	0.00 h	7.66 h	10.91 g	3.47 h	2.38 h	6.64 j	4.28 j	2.38 f	1.62 i	3.45 h	8.43 g
	40/35	8.03 f	10.63 de	0.50 d	2.89 e	6.97 f	7.99 e	4.06 f	11.22 e	15.10 d	9.64 f	4.09 f	11.05 g	7.81 f	6.35 d	4.20 f	7.55 e	13.72 d
'H1'	20/15	5.84 g	4.53 g	0.07 d	2.09 fg	4.42 g	5.29 f	3.30 g	9.37 f	12.75 e	4.64 g	2.97 g	8.70 h	6.21 g	3.87 e	2.26 h	4.90 f	10.29 e
	30/25	10.34 c	10.68 d	0.31 d	3.27 d	10.11 e	9.73 cd	3.97 f	14.90 b	18.48 a	18.93 c	5.23 e	13.73 b	11.47 b	7.46 abc	5.04 e	9.07 c	17.13 a
	40/35	10.61 b	13.21 a	1.99 c	3.95 c	11.69 b	9.84 c	5.61 d	14.60 b	18.41 a	16.53 d	5.67 d	13.09 c	11.36 b	7.86 a	9.18 c	10.50 a	17.16 a
'B1'	20/15	3.94 i	2.63 h	0.05 d	2.13 f	2.66 h	3.68 g	0.00 h	7.05 i	10.78 g	3.01 h	2.29 h	6.81 j	5.00 i	2.27 f	1.34 ij	3.19 h	7.92 h
	30/25	5.56 h	5.05 f	0.18 d	2.10 fg	4.27 g	5.17 f	0.00 h	8.59 g	11.97 f	5.05 g	2.87 g	7.97 i	5.73 h	4.20 e	2.69 g	4.43 g	9.79 f
	40/35	9.59 e	11.89 c	1.81 c	3.71 c	10.44 de	9.43 d	4.88 e	13.17 c	17.11 b	15.39 e	5.67 d	12.22 e	9.04 e	7.33 abc	7.44 d	9.55 b	16.01 b
'L2'	20/15	2.95 j	1.82 i	0.05 d	1.80 g	1.91 i	2.67 h	0.00 h	5.46 j	8.10 h	2.54 i	1.64 i	5.18 k	3.61 k	2.04 f	1.09 j	2.38 i	6.35 i
	30/25	9.94 d	10.29 e	5.38 b	8.36 b	11.06 c	10.66 b	13.40 c	12.96 c	16.33 c	23.34 b	7.01 b	12.62 d	10.48 c	7.29 bc	10.89 b	8.57 d	14.14 c
	40/35	9.92 d	10.95 d	5.38 b	8.07 b	10.67 d	10.67 b	15.10 b	12.25 d	15.34 d	23.41 b	6.08 c	11.52 f	9.37 d	7.17 c	11.04 b	8.81 cd	13.97 cd
p-value	Landraces	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Temperature	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Landrace × Temperature	<0.001	<0.001	<0.001	< 0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SED		0.121	0.181	0.224	0.148	0.169	0.143	0.301	0.192	0.190	0.223	0.150	0.147	0.160	0.260	0.199	0.138	0.151
LSD		0.252	0.376	0.464	0.307	0.351	0.297	0.624	0.399	0.394	0.462	0.312	0.304	0.332	0.540	0.414	0.287	0.313
%CV		1.9	2.7	14.9	4.2	2.8	2.3	6.5	2.1	1.6	2.1	4.1	1.7	2.4	5.8	4.2	2.5	1.5

3.3.3. Fatty Acid Methyl Esters Concentration and Composition

The landraces, temperature levels, and their interactions significantly ($p < 0.001$) affected the FAMEs in the cannabis seedlings. The concentration of FAMEs generally decreased with the increasing temperature. However, in B2 and L2, the concentrations of FAMEs were higher under 30/25 °C compared with 20/15 °C (Table 4). Amongst the FAMEs, linoleic acid, which is a polyunsaturated fatty acid, was the most abundant fatty acid in all landraces under all temperature regimes. Oleic acid, which is a monounsaturated fatty acid, was the second-highest FAME, and it was followed by palmitic acid, which is a saturated fatty acid. Notably, linoleic, oleic, and palmitic acid concentrations in landrace B2 and L2 were higher under 30/25 °C and lower under 40/35 °C compared with the other temperature levels. Whereas, linoleic, oleic, and palmitic acid concentrations increased with the increasing temperature in H1 and B1.

B2 had linoleic acid concentrations of 96.28, 107.90, and 47.74 mg/g DM under 20/15, 30/25, and 40/35 °C, with a corresponding oleic acid of 21.09, 23.52, and 9.98 mg/g DM and palmitic acid of 12.02, 14.74, and 6.97 mg/g DM, respectively. H1 showed a linoleic acid concentration of 129.61, 100.02, and 76.62 mg/g DM under 20/15, 30/25, and 40/35 °C, with a corresponding oleic acid of 25.55, 20.56, and 15.99 mg/g DM and palmitic acid of 16.48, 13.15, and 9.81 mg/g DM, respectively. B1 showed a linoleic acid concentration of 107.13, 103.46, and 87.71 mg/g DM under 20/15, 30/25, and 40/35 °C, with a corresponding oleic acid of 25.53, 23.62, and 18.90 mg/g DM and palmitic acid of 12.65, 12.12, and 12.03 mg/g DM, respectively. On the other hand, L2 had a linoleic acid concentration of 102.79, 160.26, and 67.87 mg/g DM under 20/15, 30/25, and 40/35 °C, with a corresponding oleic acid of 23.95, 38.10, and 15.83 mg/g DM and palmitic acid of 12.94, 20.73, and 9.77 mg/g DM, respectively.

Table 4. The concentration of 21 different FAMEs under three different temperature levels and four cannabis landraces (n = 3). C14 (myristic acid), C15 (pentadecaenoic acid), C16.1 (palmitoleic acid), C16 (palmitic acid), C17 (heptadecaenoic acid), C18.1 (oleic acid), C18.2 (linoleic acid), C18.3n6 (gamma-linolenic acid), C18 (stearic acid), C20.1 + C18.3n3 (cis-11-eicosenoic acid+ gamma-linolenic acid), C20.2 (eicosadienoic acid), C20.3n3 (cis-11.14.17-eicosatrienoic acid), C20.5n3 (cis-5.8.11.14.17-eicosapentaenoic (EPA)), C20 (arachidic acid), C21 (heneicosaenoic acid), C22.1 (erucic acid), C22.2 (cis-13.16-docosadienoic acid), C22 (behenic acid), C23 (tricosaenoic acid), C24.1 (nervonic acid), C24 (lignoceric acid). Means with different letters are significantly different, according to Tukey's test ($p < 0.05$). SED = Standard errors of differences; LSD 5% = least significance difference at 5 percent; % CV = percentage coefficient of variation; DM = dry mass. L2 = Ladysmith Iswazi; H1 = Durban Poison; B1 and B2 = Bergville Ugwayi wesiZulu and Natal, respectively.

		FAMEs (mg/g DW)																			
Landrace	**Temperature (°C)**	**C14**	**C15**	**C16.1**	**C16**	**C17**	**C18.1**	**C18.2**	**C18.3n6**	**C18**	**C20.1 + C18.3n3**	**C20.2**	**C20.3n3**	**C20.5n3**	**C20**	**C21**	**C22.1**	**C22.2**	**C22**	**C23**	**C24.1**	**C24**
B2	20/15	0.19 c	0.05 de	0.25 abc	12.02 e	0.14 dcd	21.09 d	96.28 h	0.87 e	6.79 c	22.70 e	0.154 bcde	0.027 g	0.085 c	1.52 bcd	0.07 bc	0.032 ef	0.038 d	0.761 f	0.095 d	0.0345 de	0.238 f
	30/25	0.18 cd	0.07 bc	0.23 bc	14.74 c	0.16 c	23.52 c	107.90 c	0.92 e	7.82 b	25.09 c	0.175 bc	0.055 b	0.037 g	1.80 b	0.09 b	0.037 b	0.044 c	1.121 b	0.134 bc	0.0381 bc	0.355 b
	40/35	0.10 g	0.04 e	0.09 e	6.97 g	0.08 e	9.98 h	47.74 l	0.44 g	3.81 g	9.11 j	0.078 f	0.032 f	0.034 g	0.83 f	0.05 c	0.035 bcde	0.084 a	0.513 g	0.065 e	0.0274 f	0.170 g
H1	20/15	0.14 ef	0.07 cd	0.24 abcc	16.48 b	0.14 cd	25.55 b	129.61 b	1.35 ab	6.84 c	29.78 b	0.178 b	0.046 cd	0.045 f	1.49 bcd	0.07 bc	0.035 bcd	0.027 g	0.776 ef	0.098 d	0.0386 bc	0.275 e
	30/25	0.23 b	0.06 cde	0.17 d	13.15 d	0.13 cd	20.56 e	100.02 g	1.19 cd	5.82 d	21.37 f	0.157 bcd	0.043 d	0.074 d	1.27 cde	0.07 bc	0.031 f	0.034 f	0.766 f	0.163 a	0.0343 de	0.288 e
	40/35	0.28 a	0.10 a	0.18 d	9.81 f	0.12 d	15.99 g	76.62 j	0.92 e	4.50 f	11.27 i	0.147 bcde	0.037 e	0.085 c	1.15 e	0.06 bc	0.034 cdef	0.034 f	1.066 c	0.117 cd	0.0368 cde	0.318 cd
B1	20/15	0.13 f	0.05 de	0.17 d	12.65 d	0.14 cd	25.53 b	107.13 d	1.14 d	7.08 c	23.98 d	0.183 b	0.045 cd	0.097 b	1.57 bc	0.14 a	0.034 cde	0.037 de	0.770 ef	0.094 d	0.0360 cde	0.291 de
	30/25	0.13 f	0.07 bcd	0.22 c	12.12 e	0.13 cd	23.62 c	103.46 e	1.28 bc	6.25 d	16.62 g	0.154 bcde	0.043 d	0.074 d	1.40 cde	0.07 bc	0.032 ef	0.038 d	0.830 d	0.095 d	0.0362 cde	0.341 bc
	40/35	0.23 b	0.12 a	0.27 ab	12.03 e	0.26 a	18.90 f	87.71 i	0.96 e	5.27 e	13.56 h	0.130 de	0.049 c	0.067 e	1.25 de	0.05 c	0.033 def	0.034 ef	1.161 b	0.129 c	0.0411 b	0.323 c
L2	20/15	0.18 cd	0.06 cde	0.176 d	12.94 d	0.15 cd	23.95 c	102.79 f	0.95 e	7.20 c	24.14 d	0.156 bcd	0.045 cd	0.084 c	1.55 bcd	0.06 bc	0.036 bc	0.036 def	0.871 d	0.096 d	0.0378 bcd	0.284 e
	30/25	0.22 b	0.09 b	0.28 a	20.73 a	0.23 b	38.10 a	160.26 a	1.43 a	10.27 a	34.63 a	0.227 a	0.073 a	0.109 a	2.48 a	0.07 bc	0.041 a	0.056 b	1.360 a	0.154 ab	0.0523 a	0.443 a
	40/35	0.16 de	0.07 bcd	0.23 bc	9.77 f	0.13 d	15.83 g	67.87 k	0.66 f	4.92 ef	11.12 i	0.125 e	0.036 ef	0.047 f	1.15 e	0.07 bc	0.033 def	0.034 ef	0.821 de	0.095 d	0.0342 e	0.262 ef
p-value	Landraces	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Temperature	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Landrace × Temperature	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	SED	0.0071	0.0050	0.01	0.137	0.0088	0.14	0.164	0.0307	0.1273	0.18	0.008	0.0012	0.00133	0.0874	0.0085	0.0	0.001	0.0144	0.0069	0.00	0.0077
	LSD	0.0147	0.0103	0.02	0.2836	0.0182	0.28	0.340	0.0636	0.2640	0.37	0.017	0.0025	0.00277	0.1813	0.0175	0.0	0.002	0.0299	0.0143	0.00	0.0160
	%CV	4.8	8.6	7.1	1.3	7.1	0.8	0.2	3.7	2.4	1.1	6.6	3.4	2.3	7.4	14.5	2.6	2.7	2.0	7.6	3.2	3.2

3.4. Multivariate Analysis

3.4.1. The Pearson's Correlation between the Germination Indexes and Biochemical Composition of Cannabis Landraces

Pearson's correlation showed no correlation between the germination indexes and the different carbohydrates ($p > 0.05$), except between the germination percentage and sorbitol, which had a weak positive correlation (r = 0.58, $p < 0.05$) (Figure 4). Notably, sorbitol also had a positive correlation with glucose (r = 0.69, $p < 0.05$). Furthermore, fructose had a strong positive correlation with glucose (r = 0.90, $p < 0.001$) and myo-inositol (r = 0.87, $p < 0.001$). Glucose also had a positive correlation with myo-inositol (r = 0.68, $p < 0.05$). Additionally, sucrose had a negative correlation with mannitol (r = −0.69, $p < 0.05$).

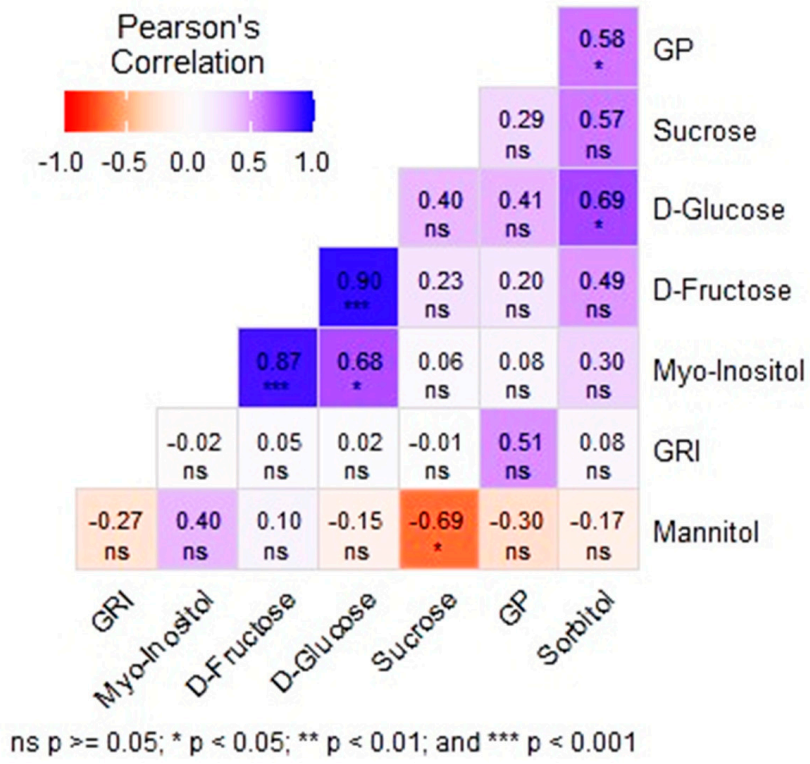

Figure 4. The Pearson's correlation between the germination percentage, germination rate index, and carbohydrates for four cannabis landraces subjected to three different temperature levels. GP (germination percentage), GRI (germination rate index).

Notably, Pearson's correlation also showed no correlation between the germination indexes and the amino acids ($p > 0.05$) (Figure 5). However, there was a strong positive correlation among the amino acids.

On the other hand, the GRI had a strong positive correlation with the FAMEs C17 (r = 0.82, $p < 0.01$), C24.1 (r = 0.75, $p < 0.01$), C20 (r = 0.72, $p < 0.01$), and C20.3n3 (r = 0.78, $p < 0.01$), as well as C24 and C22 (r = 0.76, $p < 0.01$) (Figure 6). The GRI also had a positive correlation with C16.1 (r = 0.59, $p < 0.05$), C18 (r = 0.66, $p < 0.05$), C18.1 (cis) (r = 0.63, $p < 0.05$), C16 (r = 0.62, $p < 0.05$), and C23 (r = 0.64, $p < 0.05$). The germination percentage had a positive correlation with C20.5n3 (r = 0.60, $p < 0.05$) and C20 and C18 (r = 0.66, $p < 0.05$).

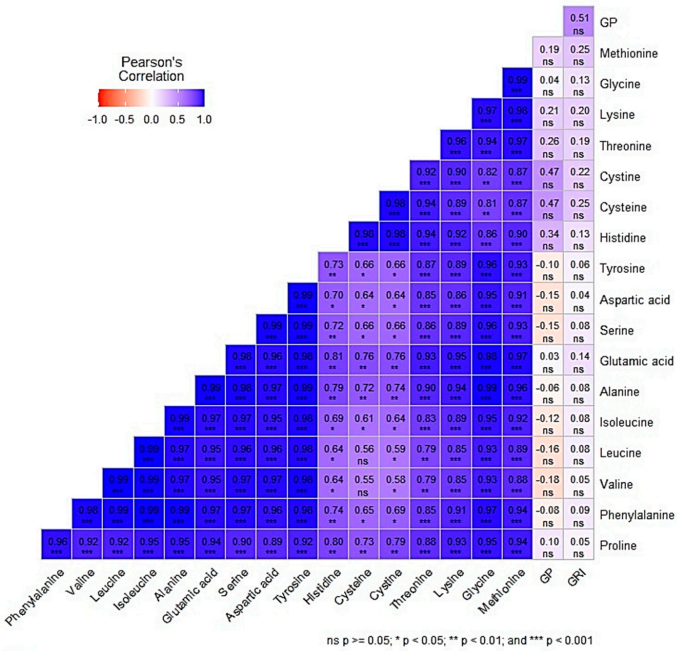

Figure 5. Pearson's correlation between the germination percentage, germination rate index, and amino acids for four cannabis landraces subjected to three different temperature levels. GP (germination percentage), GRI (germination rate index).

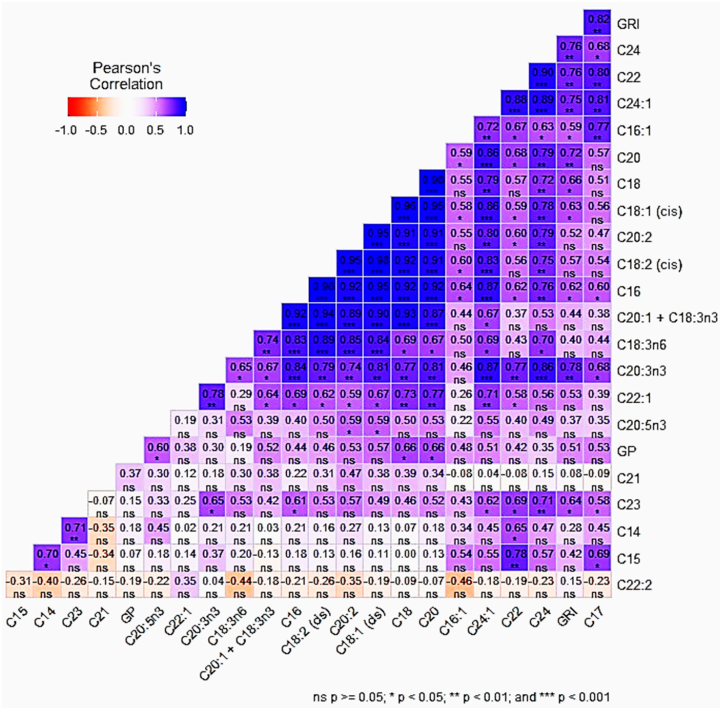

Figure 6. Pearson's correlation between the germination percentage, germination rate index, and fatty acids methyl esters (FAMEs) for four cannabis landraces subjected to three different temperature levels. GP (germination percentage), GRI (germination rate index), C14 (myristic acid), C15 (pentadecaenoic acid), C16.1 (palmitoleic acid), C16 (palmitic acid), C17 (heptadecaenoic acid), C18.1 (oleic acid), C18.2 (linoleic acid), C18.3n6 (gamma-linolenic acid), C18 (stearic acid), C20.1 + C18.3n3 (cis-11-eicosenoic acid+ gamma-linolenic acid), C20.2 (eicosadienoic acid), C20.3n3 (cis-11.14.17-eicosatrienoic acid), C20.5n3 (cis-5.8.11.14.17-eicosapentaenoic (EPA)), C20 (arachidic acid), C21 (heneicosaenoic acid), C22.1 (erucic acid), C22.2 (cis-13.16-docosadienoic acid), C22 (behenic acid), C23 (tricosaenoic acid), C24.1 (nervonic acid), C24 (lignoceric acid).

3.4.2. Principal Component Analysis for the Biochemical Composition of Cannabis Landraces

The principal component analysis was conducted separately based on different biochemical characteristics in response to temperature levels. Under carbohydrates, PCA identified two principal components (PCs), also referred to as dimensions or Dims for short throughout the study, with an eigenvalue of ≥1, which accounted for 84.90% of the total variation (Figure 7). PC1 accounted for 53.16% of the total variation and positively correlated with fructose, glucose, sorbitol, and myo-inositol. PC2 accounted for 31.75% of the total variation, and it positively correlated with mannitol and negatively correlated with sucrose.

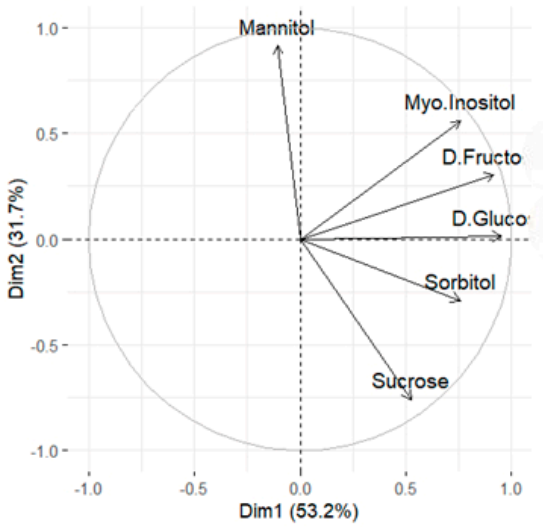

Figure 7. Principal component analysis (PCA) plot of carbohydrates in cannabis landraces subjected to different temperature regimes. D.Fructo = D-Fructose; D.Gluco = D-Glucose.

Under amino acids, PCA identified two PCs with an eigenvalue of ≥1, which accounted for 98.13% of the total variation (Figure 8). PC1 accounted for 89.09% of the total variation and positively correlated with all the amino acids. PC2 accounted for 9.04% of the total variation and positively correlated with cysteine.

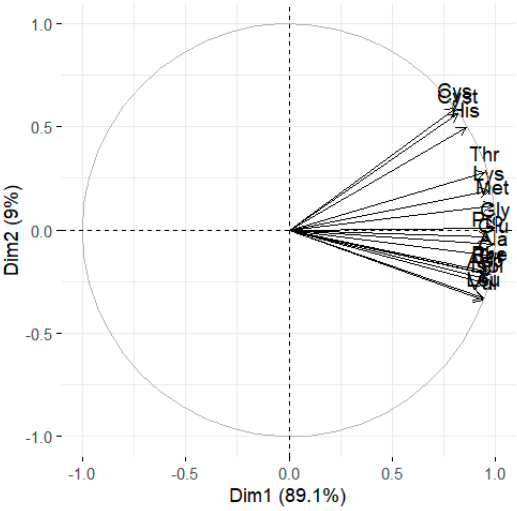

Figure 8. Principal component analysis (PCA) plot of amino acids in cannabis landraces subjected to different temperature regimes. Ala = alanine; Asp = aspartic; Cys = cysteine; Cyst = cystine; Glu = glutamic acid; Gly = glycine; His = histidine; Isol = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe = phenylalanine; Pro = proline; Ser = serine; Thr = threonine; Tyr = tyrosine; Val = valine.

Under FAMEs, PCA identified four PCs with an eigenvalue of ≥1, which accounted for 87.75% of the total variation (Figure 9). PC1 accounted for 58.71% of the total variation and positively correlated with the majority of FAMEs, including C16, C16.1, C17, C18, C18.1, C18.2, C20, C18.3n6, C20:1 + C18:3n3, C20.2, C22, C22.1, C20.3n3, C23, C24, and C24.1. PC2 accounted for 15.71% of the total variation and positively correlated with C14 and C15 and negatively correlated with C21. PC3 accounted for 8.49% of the total variation and positively correlated with C22.2 and C22.1. PC4 accounted for 4.84% of the total variation and positively correlated with C20.5n3.

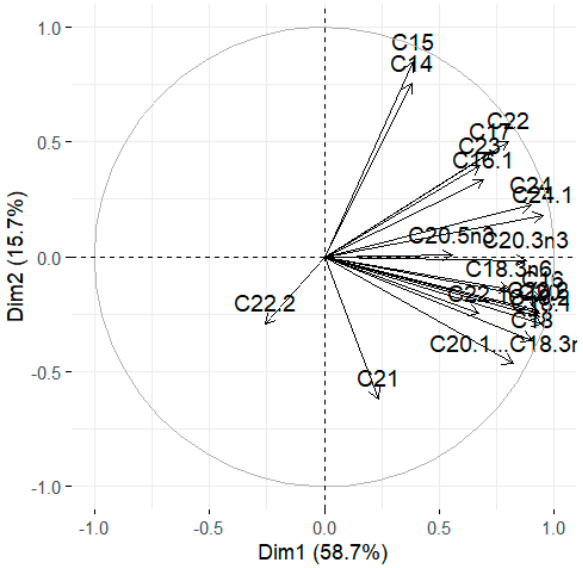

Figure 9. Principal component analysis (PCA) plot of FAMEs in cannabis landraces subjected to different temperature regimes. C14 = myristic acid; C15 = pentadecaenoic acid; C16.1 = palmitoleic acid; C16 = palmitic acid; C17 = heptadecaenoic acid; C18.1 = oleic acid; C18.2 = linoleic acid; C18.3n6 = gamma-linolenic acid; C18 = stearic acid; C20.1 + C18.3n3 = cis-11-eicosenoic acid+ gamma-linolenic acid; C20.2 = eicosadienoic acid; C20.3n3 = cis-11.14.17-eicosatrienoic acid; C20.5n3 = cis-5.8.11.14.17-eicosapentaenoic (EPA); C20 = arachidic acid; C21 = heneicosaenoic acid; C22.1 = erucic acid; C22.2 = cis-13.16-docosadienoic acid; C22 = behenic acid; C23 = tricosaenoic acid; C24.1 = nervonic acid; C24 = lignoceric acid.

4. Discussion

4.1. Seed Germination

Seed germination may vary with temperature and generally occurs within certain temperature limits, with the maximum germination efficiency occurring in a particular temperature range [4,45]. In the current study, germination varied depending on the temperature and landrace, but it generally reduced as the temperature increased. Notably, there were no variations in germination associated with the origin of the landrace. Contrary to the findings of the current study, [29] reported that the germination percentage of hemp cultivars from Canada declined at temperatures above 25 °C, while Northern European cultivars were able to withstand temperatures above 25 °C, as the germination started to decline once the temperature reached 30 °C. The findings of the current study on germination variations were associated with the landraces having optimal germination at a specific alternating temperature regime. For instance, L1, B1, M1, H1, and B2 demonstrated higher germination under 20/15 °C and lower germination under 30/25 °C. On the other hand, the B3, M2, and L2, which are landraces with the 'Iswazi' common name, had higher germination under 30/25 °C.

Generally, it is recommended that a temperature range of 15–27 °C is suitable for cannabis cultivation [26]. The authors of [27] also found that 'Georgina' and 'Victoria' hemp cultivars had the highest seed germination percentage and rate between 19 and 30 °C. Thus,

the findings of the current study corroborate with the findings by [26,27]. Additionally, [25] reported that the optimal germination temperature for the fiber hemp cultivar 'Bama' ranged between 20 and 25 °C. Furthermore, [28] reported that the germination percentage between 10 and 30 °C was not significantly different, while it declined by 20% under 35 °C compared with 30 °C. Notably, all the other landraces had the lowest germination under 40/35 °C, except for L1, B1, H1, and M1, which showed the lowest germination capacity under 30/25 °C. These findings suggest that high temperatures might have caused some damage to the seed and negatively affected the process of cell division and favor oxidative damage [46], consequently affecting the germination of seeds in cannabis landraces. The results of the current study conform with the findings by [29], who found that the germination percentage declined rapidly for all hemp cultivars as the temperature reached 40 °C and displayed limited germination capacity at temperatures near 45 °C. The authors of [28] suggested that the germination of hemp seeds is more sensitive to high temperatures compared with low temperatures. According to [46], high temperatures can reduce the germination potential of the seeds, as they may cause severe damage to the proteins, disturb their synthesis, inactivate major enzymes, and damage membranes, subsequently resulting in poor germination and stand establishment [46].

4.2. Germination Rate Index

The germination rate index varied with temperature and cannabis landrace. However, it was generally higher under 30/25 °C and lower under 20/15 °C. These findings were contrary to the germination percentage, which was generally higher under 20/15 °C and the lowest under 40/35 °C. However, the findings were associated with a temperature of 30/25 °C, speeding the initial phase of germination and shortening the germination time; whereas, a low temperature of 20/15 °C was associated with a prolonged initial phase of germination leading to the extended germination time [47]. Thus, the accelerated germination at 30/25 °C could be due to quicker seed imbibition at this temperature. Additionally, according to [48], the optimum temperature for germination rate is typically higher than the temperature required to achieve the maximum percentage of germination in partially dormant seeds. Thus, the trend of the germination percentage and rate enhanced at different temperature levels was also associated with seed dormancy, since cannabis is known to have certain residual dormancy [49,50].

4.3. Biochemical Profiling

4.3.1. Carbohydrates

Carbohydrates provide carbon sources, which are critical in regulating development and adaptation to environmental challenges [51]. The findings of the current study on the response of carbohydrates of different cannabis landraces to different temperature regimes showed that the landraces consisted of a similar composition of carbohydrates. However, the concentration of carbohydrates varied with different temperature levels and landraces. For instance, the sugar content generally decreased with the increasing temperature in B1 and L2, while it increased with the increasing temperature in H1. In B2, the content of the sugar increased with the temperature, reaching the highest under 30/25 °C and was the lowest under 40/35 °C. Nonetheless, the concentration of carbohydrates generally decreased with the increasing temperature in the cannabis landraces. The results of the current study correlated with the findings by [11], who reported that glucose, fructose, galactose, sorbose, sucrose, and xylose levels decreased with the temperature in *Ricinus communis* seedlings.

In the current study, the variations in the concentration of carbohydrates were associated with differences in adaptation mechanisms to elevated temperatures. Carbohydrates can play a protective role against stress factors, either as osmoprotectants, donors of carbon skeletons, or signaling molecules in the regulation of many defensive reactions [43]. Thus, the findings on B1, L2, and B2 were associated with the recommendation by [12] that

low-temperature stress influences sugar metabolism and sugar accumulation, while the sugars usually decrease in the warmer season.

Pearson's correlation analysis showed no significant correlations between the germination indexes and most carbohydrates. Except for sorbitol, which displayed a weak positive correlation with the germination percentage, suggesting that it may have played a role in enhancing germination; although, the effect was relatively small. Notably, sorbitol also showed a positive correlation with glucose, which also had a strong correlation with fructose, indicating that these two carbohydrates may be co-regulated during the germination process. Thus, the findings suggest that fructose and glucose may also indirectly play a role in enhancing germination. Furthermore, the first dimension (PC1) of the PCA showed a positive association between fructose, glucose, sorbitol, and myo-inositol, suggesting their collective influence on the overall metabolic landscape during germination. Hence, glucose was the most abundant carbohydrate component in the landraces, followed by fructose, while sucrose and mannitol were the least abundant sugar. This trend could be associated with sucrose being converted into glucose and fructose to support cannabis germination and seedling growth, as suggested by [52].

Generally, sucrose, glucose, and fructose are important carbon sources for germinating seeds [52]. They are fast-use substrates for respiration and adenosine triphosphate (ATP) production for growing seedlings [53–55]. Glucose is also essential in plant development and gene expression [56]. Thus, its abundance in plants was associated with various germinative processes, including germination, elongation of hypocotyls, the greening of cotyledon and expansion, and the development of true leaves [56]. On the other hand, sucrose is the translocable form of sugar required by emerging seedlings and is essential in regulating the genes involved in photosynthesis and respiration before the seedlings attain the capability for photosynthesis [52,54,57].

4.3.2. Amino Acids

The current study found that the concentration of the amino acids varied with landraces and germination temperature. For instance, the content of amino acids increased with the elevated temperature in H1, B1, and L2. Contrarily, the amino acids were the highest under 20/15 °C in B2, which then decreased to the lowest under 30/25 °C and increased under 40/35 °C. However, there was a general accumulation of amino acids, including valine, leucine, and lysine, with the elevated temperature, which was associated with the cannabis landraces responding to increased temperatures. The results of the current study corroborate the findings by [13], who reported that there was generally an accumulation of amino acids in response to the increased temperature from 28 to 38 °C by 1.4- to 3.0-fold in foxtail millet. The results of the current study also corroborate the findings by [11], who reported that the levels of almost all identified amino acids in *Ricinus communis* seedlings were higher at 35 °C compared with 20 °C.

Additionally, Pearson's correlation analysis showed no significant correlations between the germination indexes and the amino acids. However, there was a strong positive correlation among the amino acids themselves, indicating a tightly regulated network of amino acid metabolism during germination. On the other hand, the PCA showed that the first principal component collectively explained the majority of amino acid variation at 89.09%. These findings conform with the recommendation that amino acids play a role as osmoprotectants to resist abiotic stress by increasing the content of certain amino acids [58,59]. Generally, the increase in amino acids and some carbohydrates at higher temperatures is associated with their role in stabilizing membranes and maintaining cellular osmotic pressure [14]. Furthermore, [60] suggested that different plant species differ in their capacity to accumulate specific amino acids under abiotic stress conditions, with proline being the most predominantly accumulated in various species grown in saline conditions. In the current study, the cannabis landraces generally accumulated the essential amino acids, such as leucine, lysine, and valine, with the increasing temperature. Thus, the accumulation of these essential amino acids was associated with the response of cannabis

landraces to the elevated temperature. This is because metabolic processes, such as the catabolism of lysine via the saccharopine pathway, primarily drive lysine involvement in numerous forms of the plant stress response, demonstrating a significant influence on both abiotic and biotic stress responses [61].

4.3.3. Fatty Acid Methyl Esters

Fatty acids play a critical role in seed germination and seedling development, both physiologically and anatomically [62,63]. In the current study, the content of FAMEs varied with landrace and temperature. For instance, the content of FAMEs decreased with the increasing temperature in H1 and B1, while it increased under 30/25 °C compared with 20/15 °C in B2 and L2. However, the content of FAMEs was generally the highest under 30/25 °C and lowest under 40/35 °C. Notably, the germination rate was also generally the highest under 30/25 °C. Additionally, Pearson's correlation analysis revealed that the germination percentage and germination rate index had strong positive correlations with several FAMEs, indicating a strong relationship between lipid composition and seed germination performance. The PCA results also revealed that PC1 represented a wide range of FAMEs associated with optimal germination performance, explaining most of the variation and showing a positive connection with a variety of FAMEs. Therefore, the findings of the current study on the increased content of FAMEs under 30/25 °C were associated with energy metabolism in seeds, reflecting the role of lipids as energy sources in seeds during embryo development [14].

On the other hand, the reduced content of FAMEs with the elevated temperatures of 40/35 °C was associated with temperature regulating some of the growth chemical activity responsible, such as lipid composition, for the cannabis landraces to adapt under elevated temperature during germination. Temperature generally influences the amount of oxygen available, making it less soluble in the cytoplasm as the temperature increases, thereby directly affecting the lipid profile by destabilizing the desaturase enzyme [64,65]. Oxygen enables the resumption of respiration and reactivation of metabolism during seed imbibition, leading to the production of reducing power and ATP required for seed germination [66,67]. However, the oxygen requirement for seed germination varies based on the species, dormancy level, and temperature [66].

According to [15], many plants self-regulate the lipid composition to maintain membrane fluidity at different temperatures to maintain structural integrity. A low temperature is known to result in membrane rigidification by inducing the close packing of membrane lipids, while a high temperature leads to fluidization [16,17,68]. Thus, the membrane fluidity in plants decreases and increases to adapt to low and high temperatures, respectively [68].

The seedlings of cannabis landraces were composed of saturated, monounsaturated, polyunsaturated, and unsaturated fatty acids. Linoleic acid was the most abundant FAME, followed by oleic and palmitic acid in all landraces under all temperature levels. The results of the current study corroborate the findings of [15], who reported that EPA content decreased with the increasing temperature in *Nannochloropsis salina*. It is suggested that a high proportion of polyunsaturated fatty acids helps maintain membrane fluidity under low temperatures, thereby improving seed performance [8]. Hence, the germination of cannabis landraces was generally higher under 20/15 °C compared with 30/25 and 40/35 °C. Furthermore, the abundance of linoleic acid and oleic acid conforms with the recommendations that cannabis seed oil comprises 90% polyunsaturated fatty acids and desirable quantities of omega-6 and omega-3 lipids [69].

It is worth noting that this study focused on seed germination and seedling growth, while temperature impacts multiple stages of plant growth and development, influencing the morphology, anatomy, and physiology of cannabis. For instance, high temperatures can accelerate maturity by inducing flowering and result in stunted growth [26]. Temperature also affects net photosynthesis (PN): low temperatures slow PN, while excessive heat stops it, forcing plants to use up energy on cooling through water uptake and transpiration [70].

While this study focused on the effect of temperature on germination, other factors such as moisture, seed dormancy, seed quality, and light also play significant roles [71,72]. This highlights the need for further research to optimize cannabis cultivation under varying environmental conditions, which will provide a more comprehensive understanding of how to improve cultivation practices in diverse environments.

5. Conclusions

The current study highlights the effect of temperature on the seed germination and seedling biochemical factors of cannabis landraces. It was concluded that cannabis landraces have superior germination under specific alternating temperatures, but notably, elevated temperature decreased the germination of cannabis landraces. L1, B1, H1, B2, and M1 exhibited higher germination rate indexes under the cooler conditions of 20/15 °C, while landraces B3, M2, and L2 thrived under the warmer temperatures of 30/25 °C. Additionally, it was concluded that germination temperature affects the biochemical factors during cannabis germination and seedling development. This was attributed to the observed decrease in sugars and FAMEs and increase in amino acids, which was associated with the adaptation mechanism of cannabis landraces towards the elevated temperatures of 40/35 °C. The findings of the current study, therefore, highlight the complex relationship between temperature and biochemical composition in cannabis germination. This knowledge can help growers make better decisions concerning the planting season for achieving a superior seedling establishment, which might translate to a better harvest. Furthermore, this knowledge will be useful when selecting landraces better suited for specific regions and in developing heat-resistant genotypes, which is crucial given that heat stress has become very common in recent years. Based on the current findings, it is recommended that further research should assess the effect of different temperature regimes, particularly elevated temperatures, on physiological and biochemical processes, including changes in structure and storage substances during seed germination.

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