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ORIGINAL ARTICLE

Genetic structure and responses of flax (Linum *usitatissimum* L) germplasm to phytotoxic aluminum (Al^{3+}) in acid soils of southern Chile

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ABSTRACT
Flax (*Linum usitatissimun* L) is a versatile crop valued for its oil and functional compounds. However, the presence of phytotoxic aluminum (Al^{3+}) in acid soil, limits its productivity, specifically in southern Chile where acid soils of the Andisol type abound. The objective of this study was to characterize the genetic structure of 40 flax accessions using 2,200 single nucleotide polymorphism (SNP) markers using STRUCTURE and Neighbor Joining (NJ) algorithms, and to analyze their shoot and root phenotypic responses to aluminum toxicity in an acid soil to identify tolerant genotypes that could be used in flax breeding. STRUCTURE and NJ methods grouped the 40 genotypes into three groups. Significant treatment and genotypic effects were observed for total root length (TRL), root area (RA), apical root length (ARL) and root volume (RV) ($p < 0.05$). STRUCTURE groups 1 and 2 (G1 and G2) outperformed group 3 (G3) for RA (control and Al^{3+}), ARL (Al^{3+}), TRL (control), RV (control and Al^{3+}), and relative root area. Fisher exact test for root traits under control and Al conditions enabled the identification of genotypes differentially responding to stress and non-stress treatments. Pearson´s correlation analysis indicated that root traits either under Al^{3+} stress or limed soil conditions exhibited moderate to high associations among them (r= 032 – 096), suggesting shared root development regulation. Hierarchical and multivariate analyses of root traits and their relative growth indices identified the Al^{3+} tolerant genotypes G188 (fiber type), G87 (fiber type), and G401 (oil type), which showed superior relative total root length (RTRL), relative apical root length (RARL), and relative root area (RRA). The present study revealed unexplored genetic variation in response to Al^{3+} stress and provide potential parental lines to improve flax adaptation to acidic soil conditions as those prevailing in southern Chile.

Keywords: Flax, Population structure, Aluminum toxicity, Acid soil, Root traits, Aluminum tolerance.

1. Introduction

Acidic soils pose a major constraint to agricultural productivity in southern Chile These soils, primarily derived from volcanic ash, exhibit high levels of structural aluminum and are widespread from the Maule Region southward (Luzio, 2010) Approximately, 40% and 70% of soils in the IX and X Regions, respectively, show aluminum saturation levels above 5% (CIREN, 2003) In soils with pH below 55, aluminum becomes solubilized primarily as $Al(OH)_3$ and Al^{3+}, the latter being highly phytotoxic Soluble Al^{3+} can penetrate root cells, inhibiting root elongation, limiting the root system's capacity for soil exploration, and consequently reducing water, nutrient uptake, and ultimately crop yield (Kochian et al., 2005; Luzio and Casanova, 2020).

In response, plants have evolved two principal molecular strategies for aluminum resistance: (i) the exclusion of Al^{3+} from root tissues via chelation in the rhizosphere, and (ii) internal tolerance, involving sequestration and compartmentalization of Al^{3+} within cellular organelles such as vacuoles (Zhang et al., 2019; Kochian et al., 2015).

Organic acid exudation is a well-characterized exclusion mechanism, whereby roots release compounds such as citric, malic, and oxalic acids that chelate Al^{3+}, preventing its uptake These responses are regulated by specific transporter genes, including *Hordeum vulgare aluminum-activated citrate transporter 1* (*HvAACT1*) in barley and *Triticum aestivum Al-activated malate transporter 1* (*TaALMT1*) in wheat, which encode citrate and malate transporters, respectively (Furukawa et al., 2007; Sasaki et al., 2004) The heterologous expression of these genes has conferred Al^{3+} tolerance in multiple crop species including barley, wheat, canola, rye, and soybean (Delhaize et al., 2004; Palmer et al., 2016; Ramesh et al., 2018) In addition to transporter-mediated responses, aluminum stress induces oxidative damage characterized by reactive oxygen species (ROS) accumulation and lipid peroxidation, triggering antioxidant defense systems to maintain redox balance (Xu et al., 2012; Tripathi and Agrawal, 2013) Given that Al^{3+} is among the most limiting factors for plant development in acidic soils, there is a critical need to identify crop species and genotypes capable of thriving under these conditions (Heidarabadi et al., 2011).

Flax (*Linum usitatissimum* L) is a historically important crop used for both oil (linseed) and fiber (flax) production. In 2022, linseed global production reached 39 million tons with Russia, Kazakhstan, and Canada ranked as the main producer (FAOSTAT, 2023) In the same year, flax production was approximately 897,000 tons, with France, Belgium, and Belarus being the top producers (FAOSAT, 2023).

Linseed is valued because of its high-quality oil, and functional dietary metabolites including alpha linolenic acid, proteins, mucilage, and lignans (Singh et al., 2011; Soto-Cerda et al., 2018). However, achieving higher linseed yield potential is hindered because like many crops, linseed is sensitive to Al^{3+} toxicity (Diemitriev et al., 2016). Nonetheless, recent research has revealed considerable genetic variability in Al^{3+} tolerance within the species, including cultivars exhibiting enhanced resistance (Dmitriev et al., 2016). Therefore, identifying and

characterizing Al^{3+}-tolerant linseed genotypes is essential for adapting linseed production to acid soils, while maintaining high yield and seed quality (Berti et al., 2010).

Table I

List of forty flax (*L usitatissimum*) genotypes used for Al tolerance assessment.

N°	Internal code	*Name	Origin
1	G13	O_HUN_C_CN98276	Hungary
2	G16	O_HUN_C_CN98263	Hungary
3	G18	O_IND_C_CN98250	India
4	G25	U_MAR_C_CN98193	Morocco
5	G26	O_IRL_C_CN98192	Ireland
6	G30	O_IND_C_CN98135	India
7	G32	O_URY_C_CN98100	Uruguay
8	G37	O_ARG_C_CN98039	Argentina
9	G40	O_ARG_C_CN98027	Argentina
10	G43	O_ARG_C_CN98007	Argentina
11	G52	O_DEU_C_CN97886	Germany
12	G81	O_CAN_C_CN97571	Canada
13	G87	F_RUS_L_CN97503	Russia
14	G100	O_DEU_B_CN97430	Germany
15	G124	O_HUN_C_CN97287	Hungary
16	G134	O_PAK_C_CN97103	Pakistan
17	G151	O_CAN_C_CN52732	Canada
18	G158	O_USA_C_CN33399	USA
19	G160	F_UNK_C_CN33393	Unknown
20	G162	O_CAN_C_CN33389	Canada
21	G167	F_RUS_C_CN32542	Russia
22	G177	O_CAN_C_CN19004	Canada
23	G178	O_CAN_C_CN19003	Canada
24	G188	F_FRA_C_18986	France
25	G216	O_CAN_CDC Bethune	Canada
26	G221	O_CAN_B_CN101560	Canada
27	G225	O_CAN_B_CN101496	Canada
28	G234	F_CHN_B_CN101416	China
29	G237	F_RUS_U_CN101402	Russia
30	G240	F_TUR_U_CN101386	Turkey
31	G263	O_CAN_B_CN 101598	Canada
32	G285	F_RUS_U_101396	Russia
33	G290	F_TUR_U_CN101382	Turkey
34	G292	F_UKR_U_CN101378	Ukraine
35	G307	O_GBR_C_CN101265	Great Brittain
36	G322	O_MAR_B_CN101026	Morocco
37	G343	O_ROM_C_CN100674	Romania
38	G398	O_CL_B_C398	Chile
39	G401	O_CL_B_C401	Chile
40	G500	O_NLD_C_Bilstar	Holland

*O: oil, F: fiber, C: cultivar, B: breeding material, L: landracea, U: unknown

The objective of this study is to evaluate aluminum stress tolerance in 40 *L usitatissimum* genotypes (linseed and flax morphotypes) at the seedling stage in an acid soil with 16% Al saturation through the assessment of root and shoot traits, and their association with Al^{3+} tolerance. The identification of Al tolerant genotypes will provide genetic diversity to support breeding efforts towards enhancing linseed adaptation and competitiveness in the acidic soils of southern Chile.

2. Material and Methods

2.1. Flax germplasm

A diverse panel of 40 flax accessions including both morphotypes (oil and fiber) was assembled from the Canadian flax core collection (Diederichsen et al., 2013), including two Chilean advanced breeding lines. The association panel includes 25 cultivars, 8 breeding lines, 1 landrace and 5 accessions of unknown improvement status grouped into 29 oil, 10 fiber, and 1 unknown morphotype (Table I).

2.2. Genotyping and genetic structure analysis

Single nucleotide polymorphism (SNP) data of the entire flax core collection (n = 407) was initially generated after resequencing all accessions using Illumina HiSeq 2000 platform in 100 bp paired-end mode at an average coverage of 17X, generating 1.7 million SNPs. Further data curation including the removal of SNPs in long terminal repeat regions, generated a set of 570,443 SNPs, 20% missing data rate. From the core data set, SNP information was extracted for the selected 40 accessions resulting in 2,218 SNPs evenly distributed across the 15 pseudomolecules of flax (You et al., 2018) characterized by a genotyping rate > 99% and a Minor Allele Frequency (MAF) > 0.05.

Genetic structure was studied using the 2,218 SNPs in STRUCTURE v.2.3.4 (Pritchard et al., 2000). The number of sub-groups was determined using the web-based software Structure Harvester (http://taylor0.biology.ucla.edu/structureHarvester/), which is based on the Evanno method (Evanno et al., 2005). Neighbor-joining (NJ) phylogenetic analysis was performed based on the 2,218 SNPs using TASSEL v 5.2.31 (Bradbury et al., 2007).

2.3. Soil Collection and Preparation

The study was conducted using an Andisol soil from the Cunco series, which is representative of one of the main linseed cultivation zones in the La Araucanía Region, Chile. A total of 300 kg of soil was collected from the "Avícola Huichahue" farm, located at Faja 24,000, Cunco, La Araucania Region, Chile. Soil sampling was performed using a systematic sampling method over a surface area of 2500 m^2.

The collected soil was transported to the Soil Laboratory of the Universidad Católica de Temuco, where it was homogenized, air-dried at room temperature, and sieved through a 5 mm mesh. Approximately 1 kg of this composite soil sample was sent to the laboratory for analysis of aluminum saturation and its nutritional profile (Table II). Based on the results of the soil analysis, the bulk soil was divided into two

fractions. One fraction was stored as is, while the second was incubated for four weeks with 2 g of $CaCO_3$ per kg of soil. The soil was mixed once per week and kept in the dark during the incubation period, covered with black polyethylene to ensure appropriate conditions. After incubation, one kg of soil was analyzed to determine the residual aluminum saturation (Table II).

Table II

Chemical properties of the Andisol utilized in soil-based assay.

Variable	Control	With application of $CaCO_3$
N, mg kg^{-1}	36	37
Olsen P, mg kg^{-1}	29	19
K, mg kg^{-1}	148	208
pH (H_2O)	4.81	5.67
Organic matter (%)	19.86	19.76
Exchangeable Ca, $cmol_{(+)}$ kg^{-1}	1.98	10.0
Exchangeable Mg, $cmol_{(+)}$ kg^{-1}	0.29	0.56
Exchangeable Na, $cmol_{(+)}$ kg^{-1}	0.11	0.10
Exchangeable K, $cmol_{(+)}$ kg^{-1}	0.38	0.53
Exchangeable Al $cmol_{(+)}$ kg^{-1}	0.529	0.067
Aluminum saturation (%)	16.08	0.6
ECEC, $cmol_{(+)}$ kg^{-1}	3.29	11.26
Bases sum, $cmol_{(+)}$ kg^{-1}	2.76	11.19

ECEC: Effective cation exchange capacity

2.4. Experimental setup and phenotyping

Four seeds per genotype were sown in polyvinyl chloride (PVC) pots with a volume of 294 cm^3. Plants were grown under controlled environmental conditions 22/16 °C, photoperiod of 16/8 h (light/dark), relative humidity of 40–60%, and light intensity of 400 µmol m^{-2} s^{-1}. Irrigation was applied every 48 hours using 10 mL of distilled water per pot. Fourteen days after germination, the seedlings were thinned to maintain three homogeneous plants per pot. To assess the effect of phytotoxic aluminum (Al^{3+}) on root and shoot growth, the 40 flax genotypes were evaluated over a 14-day period under contrasting soil conditions with and without Al^{3+}. Following the treatment period, pots were submerged in water to gently remove soil and release the root systems without damage and scanned.

Roots were scanned using a high-resolution root scanner (LA2400, Epson 11000XL, Long Beach, CA, USA). The root images were analyzed using WinRHIZO software (Reagent Instruments, Montreal, QC, Canada) to obtain the total root length (TRL, cm), root area (RA, cm^2), root diameter (RD, mm), root volume (RV, cm^3), and root tips (RT). In addition, the apical root length (ARL, cm) was measured from the root base at the stem junction to the tip of the longest root within the system. After scanning, both roots and aboveground tissues (stems and leaves) were dried in an oven at 65 °C for 48 hours to determine the plant dry weight (PDW, mg), root dry weight (RDW, mg), and shoot dry weight (SDW, mg) using an analytical balance. These data were used to calculate the relative growth index for each trait defined as the ratio: (Trait value under Al^{3+} treatment / Trait value under control conditions).

2.5. Experimental design and statistical analysis

The experiment followed a completely randomized design (CRD) with three replicates per genotype under two treatments (presence and absence of Al^{3+}). All phenotypic data were analyzed using a two-way analysis of variance (ANOVA) at a 5% significance level in GenStat software version 18 (VSN International, 2015). Pearson correlation coefficients (r) were calculated between significantly affected traits using the R package "ggplot2" (Wickham, 2016). A hierarchical clustering analysis was performed using the online platform Morpheus (https://software.broadinstitute.org/morpheus/), applying Euclidean distance as the metric and average linkage as the clustering method.

3. Results and discussion

3.1. Population structure

The Bayesian-based clustering approach implemented in STRUCTURE identified three major groups according to the Evanno method (Figure 1A). Group 1 (G1, red) contained four accessions of the fiber morphotype (i.e. F_RUS_L_CN97503, F_UNK_C_CN33393, F_FRA_C_18986, and F_RUS_U_101396) of diverse geographic origin. Group 2 (G2, blue) included 27 accessions, mostly of the oil morphotype from all geographic origin considered in this panel, while group 3 (G3, green) contained four accessions of both morphotypes that showed admixture alleles from the three groups.

The phylogenetic analysis using the NJ algorithm also partitioned the 40 accessions into three major groups, largely corresponding to the same major groups identified by STRUCTURE (Figure 1B). In G1 of the NJ tree, only the accession F_RUS_L_CN97503 was not clustered in G1 as observed in the STRUCTURE panel. Similarly, the NJ tree clustered together 24 out of the 27 accessions included in G2 by STRUCTURE analysis. On the other hand, G3 of the NJ dendrogram grouped the same five accessions as those included by STRUCTURE algorithm, but additionally clustered other four genotypes. Overall, our combined population structure analyses were consistent with clustering patterns obtained by STRUCTURE and NJ algorithms, despite their differences in mathematical algorithms, and both analyses provide valuable information to assist in the decision-making process in flax breeding programs.

The level of population structure in a species has implications for the design of crosses in breeding programs (Newell et al., 2010). Flax is a predominantly self-pollinated crop with limited natural gene flow. Our results confirmed the presence of substantial genetic structuring across flax groups in line with their reproduction system and improvement status, where most of the lines are either cultivars or breeding materials, which has undergone genetic diversity erosion

during domestication and breeding (Uysal et al., 2010). Genetic structure assessment of 383 flax accessions representing 37 flax growing countries resulted in a total of four major groups, and the grouping patterns were influenced by environmental and anthropogenic selections. Thus, understanding genetic divergence and geographic origins of germplasm in line with their cultivation history are essential for conservation and breeding (Sertse et al., 2019; Hoque et al., 2020).

Figure 1.

A) Model-based population structure of 40 flax accessions belonging to three groups predefined by the STRUCTURE software. **B)** Neighbor-joining (NJ) tree of 40 flax accessions based on the 2,218 SNP markers.

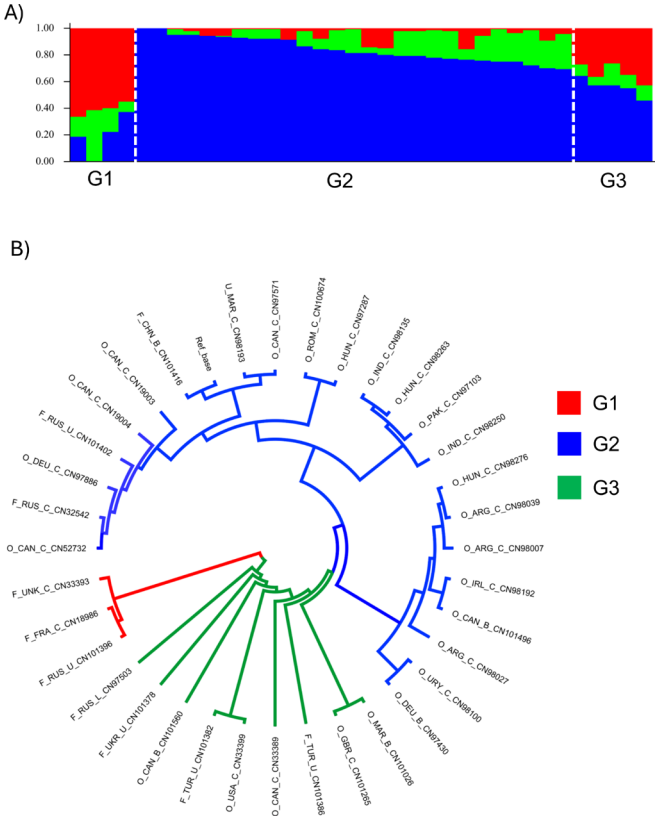


Table III

Analysis of variance for root and shoot traits assessed in 40 flax genotypes under aluminum contrasting soil conditions.

Trait	Treatment (T)	Genotype (G)	T x G
RA (cm^2)	3.574^{**}	4.200^{***}	$0.471^{n.s}$
RV (cm^3)	$0.0003^{*.}$	0.0003^{***}	$0.0000^{n.s.}$
RD (mm)	$0.013^{n.s.}$	0.027^{***}	$0.006^{n.s.}$
ARL (cm)	33.080^{**}	17.484^{***}	$3.962^{n.s.}$
TRL (cm)	312.91^{***}	356.13^{***}	$31.98^{n.s.}$
RT	$1468.2^{n.s}$	1307.2^{**}	$231.8^{n.s.}$
DPW (mg)	$0.21^{n.s.}$	131.19^{***}	$60.08^{n.s.}$
DRW (mg)	$0.094^{n.s}$	8.951^{***}	$2.580^{n.s}$
DSW (mg)	$0.59^{n.s.}$	91.56^{***}	$54.24^{n.s.}$
R/S	$497.0^{n.s.}$	797.0^{***}	605.4^{*}

*p <0.05; **p < 0.01; ***p < 0.001; n.s. = non significant.

Table IV

Phenotypic variability of four root traits evaluated under aluminum saturation and limed soil conditions.

Trait	Treatment	*n*	Mean	Standard deviation	Range	C.V. (%)
TRL (cm)	Aluminum	240	25.69	7.70	9.44 - 40.69	29.97
	Control	240	27.97	8.36	11.79 - 48.03	29.91
ARL (cm)	Aluminum	240	9.52	1.88	5.59 - 12.91	19.79
	Control	240	10.26	1.89	6.50 - 13.81	18.50
RA (cm^2)	Aluminum	240	3.06	0.86	1.36 - 4.81	28.33
	Control	240	3.30	0.89	1.50 - 5.31	27.18
RV (cm^3)	Aluminum	240	0.03	0.008	0.02 - 0.05	26.90
	Control	240	0.031	0.008	0.018 - 0.047	26.13

TRL: Total root length; ARL: Apical root length; RA: Root area; RV: Root volume.

3.2. Root traits variation

Analysis of variance showed significant treatment and genotype effects for TRL, RA, ARL, and RV (Table III). Aluminum stress markedly reduced root growth across genotypes. Mean values for all traits were higher under the control compared with the aluminum treatment (Table IV), confirming the inhibitory effect of Al^{3+}. Similar responses have been reported in soybean (Kopittke et al., 2015), wheat (Pereira, 2018), and other agriculturally important crops (He et al., 2019; Jiang et al., 2022), where Al^{3+} rapidly inhibits auxins transport and cell wall integrity, limiting root elongation. The consistent reduction in root traits observed here confirms that root inhibition is a sensitive indicator of aluminum toxicity in flax (Dmitriev et al., 2016).

The coefficients of variation for TRL, ARL, RA, and RV ranged from 18.5% to 29.9% in control and from 19.8% to 30.0% under aluminum stress (Table IV). Among traits, ARL displayed the lowest variation, suggesting limited genetic diversity for apical root initiation. In contrast, TRL showed the highest variability, identifying it as the most informative individual trait for distinguishing genotypic responses. These findings suggest that root elongation traits may serve as effective phenotypic markers for aluminum tolerance in linseed.

The differential responses observed highlight the existence of genotypes capable of maintaining superior root development under stress. Such genotypes represent valuable material for breeding programs aimed at enhancing flax adaptation to acidic soils (Dmitriev et al., 2016; Hoque et. al., 2020). Given the high variability detected in TRL, this trait emerges as a promising selection criterion for screening germplasm. However, the integration of these phenotypic

evaluations with relative growth indices analyses could help in fine-tuning the identification of superior flax genotypes suitable for improving aluminum tolerance.

Figure 2.

Variation of the relative root area (RRA), relative total root length (RTRL), and relative apical root length (RARL) indices in 40 *L. usitatissimum* genotypes evaluated under contrasting Al^{3+} soil conditions.

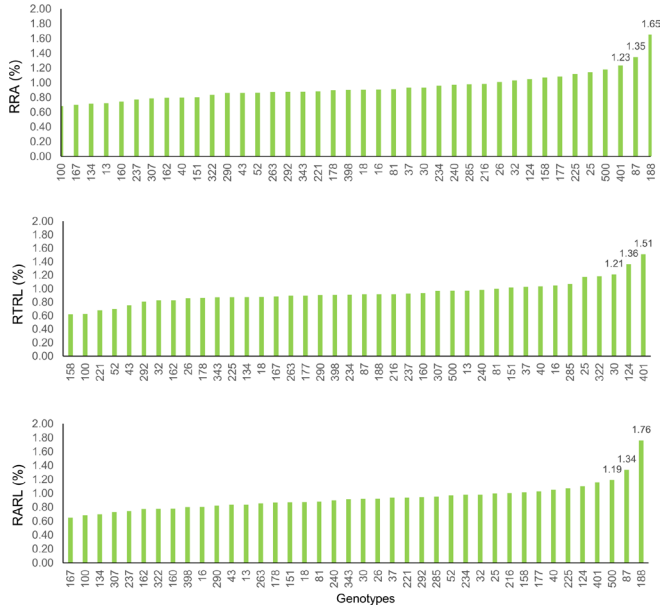


Table V

Comparison of root traits among STRUCTURE groups evaluated under aluminum saturation and limed soil conditions.

Trait	G1	G2	G3	P
RA_Al	3.10[a]	3.24[a]	2.24[b]	0.035*
RA_C	2.85[a]	3.62[ab]	2.51[ac]	0.016*
ARL_Al	8.44[a]	10.15[ab]	7.54[ac]	0.015*
ARL_C	8.76[a]	10.83[a]	9.83[a]	0.068
TRL_Al	26.56[a]	27.25[a]	18.79[a]	0.084
TRL_C	24.09[a]	30.81[ab]	20.88[ac]	0.029*
RV_Al	0.028[a]	0.031[ab]	0.022[ac]	0.017*
RV_C	0.027[a]	0.034[ab]	0.025[ac]	0.020*
RRA (%)	96.95[a]	96.96[a]	78.36[b]	0.023*
RARL (%)	124.68[a]	90.77[a]	94.82[a]	0.411
RTRL (%)	120.78[a]	89.01[a]	89.97[a]	0.341
RRV (%)	113.54[a]	92.02[a]	88.51[a]	0.387

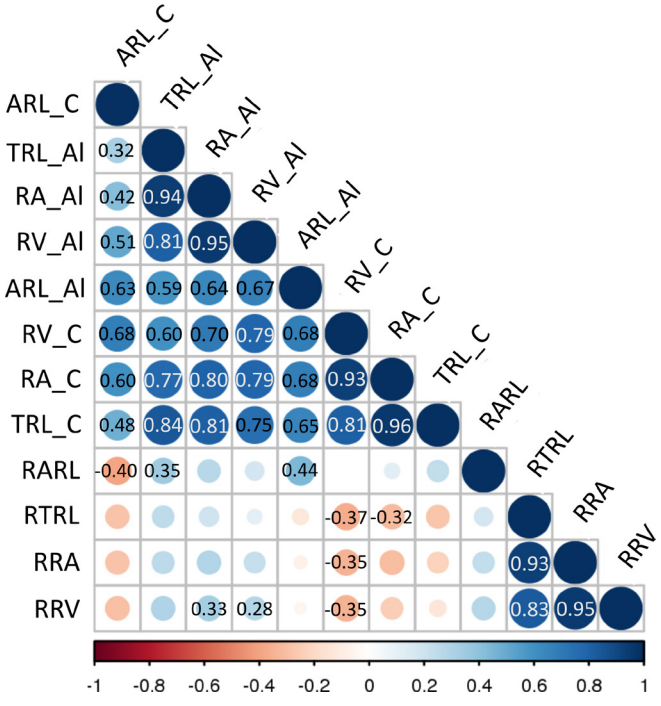
*p < 0.05. RA: Root area, ARL: Apical root length, TRL: Total root length, RV: Root volume, C: Control treatment, Al: Aluminum treatment, RRA: Relative root area, RARL: Relative apical root length, RTRL: Relative total root length, RRV: Relative root volume. Different letters indicate statistically significant differences.

The relative growth index effectively discriminated flax genotypes differing in aluminum (Al^{3+}) tolerance. Genotypes with a relative growth index > 1.0 exhibited superior stress responses, with minimal impact on root traits. For relative root area (RRA), G188, G87, and G401 performed best, while G100, G167, and G134 were most affected (Figure 2). For relative total root length (RTRL), G401, G124, and G30 were superior, whereas G158, G100, and G221 showed poor performance, responding favorably only under control conditions. For relative apical root length (RARL) G188, G87, and G500 were the most stable, contrasting with G167, G100, and G134. Overall, G188 emerged as the most stable across traits, particularly for RRA and RTRL, while G401 was superior for RARL (Figure 2). When root traits were compared among STRUCTURE groups, group 1 and 2 outperformed group 3 for RA (control and Al^{3+}), ARL (Al^{3+}), TRL (control), RV (control and Al^{3+}), and relative root area (Table V). These results suggest that allele frequency distribution and flax breeding history could have favored superior root traits under aluminum toxicity. However, more evidence using larger population will be needed in the future.

Al^{3+} toxicity is known to impair root growth within hours of exposure by targeting root apices, leading to reduced elongation, increased lateral branching, and thickening (Ryan et al., 1993; Sivaguru and Horst, 1998; Hoque et. al., 2020). These responses were consistent with the genotypic variability observed. Pearson correlations confirmed 40 significant associations ($p < 0.05$), mostly positive, though negative correlations between relative growth indices and control traits highlighted trade-offs between stability and performance in non-stressed conditions (Figure 3).

Figure 3.

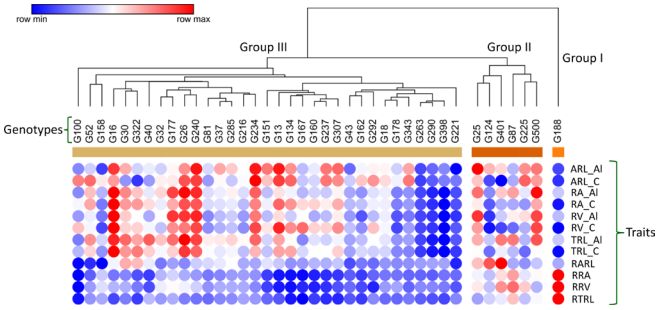
Pearson's correlation between root traits evaluated under contrasting Al^{3+} soil conditions and their respective stability indices.



ARL: Apical root length, TRL: Total root length, RA: Root area, RV: Root volume, C: Control treatment, Al: Aluminum treatment, RRA: Relative root area, RTRL: Relative total root length, RARL: Relative apical root length, RRV: Relative root volume. Cells with values correspond to statistically significant correlations ($p < 0.05$).

Figure 4.

Hierarchical clustering of 40 flax accessions using root morphological traits and their corresponding relative growth indices assessed under contrasting Al^{3+} soil conditions. Color rule indicates the strength of the correlations between the different variables evaluated.



Aluminum tolerance in flax likely involves multiple mechanisms. Mechanisms well documented in other plant species include organic acid exudation (citrate, malate, oxalate) from root apices chelates Al^{3+}, maintaining auxin transport and root elongation (Sun et al., 2010; Chauhan et al., 2021). At the physiological level, Al triggers oxidative stress, including lipid peroxidation and altered antioxidant enzyme activity (Cakmak and Horst, 1991; Yamamoto et al., 2001). In flax, transcriptomic studies show induction of glutathione S-transferase (GST) genes in resistant flax cultivars, contributing to antioxidant defense and cell wall lignification (Dmitriev et al., 2016).

Heatmap clustering grouped the 40 genotypes into three clusters: Group I (G188) with the highest relative growth indices; Group II (G25, G124, G401, G87, G225, G500) with moderate-to-high root performance; and Group III, comprising the remaining 33 sensitive genotypes (Figure 4). Notably, G188 (Hermes, a French fiber flax) is recognized as an Al^{3+}-tolerant cultivar widely used in genetic studies (Zyablitsin et al., 2018; Krasnov et al., 2019). G87 (fiber flax from the former Soviet Union) and G401 (a Chilean oil-type line) also showed consistent tolerance.

In conclusion, the evaluated germplasm revealed significant genetic variability for Al^{3+} tolerance in flax. Genotypes G188, G87, and G401 represent promising parental material for breeding programs, combining tolerance with desirable agronomic traits. This germplasm also provides a foundation for physiological and molecular studies aimed at elucidating Al^{3+} tolerance mechanisms in flax.

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