Genome-wide association and genomic prediction of root system architecture in alfalfa.
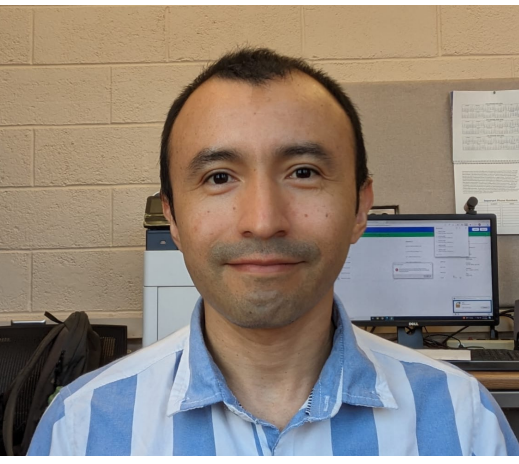
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Introduction

- Alfalfa root system architecture (RSA) influences biomass yield, capture of water, storage of nutrients, soil stabilization, and nitrogen fixation (Lynch, 2022).

- DArTag genotyping allows the identification of molecular markers associated with root morphological traits and the implementation of genomic selection to enhance the alfalfa adaptation to adverse environmental conditions.

- Our goal was to identify molecular markers associated with four traits of RSA and to test the genomic BLUP model (GBLUP) to find the high-merit genotypes for RSA.

Plant materials

Table 1. Summary of root system architecture (RSA) populations, with the number of plants phenotyped and genotyped (P&G), or just genotyped (Geno) (Bucciarelli et al., 2021). HF-B, high fibrous & branched root; LF-T, low fibrous & taproot.

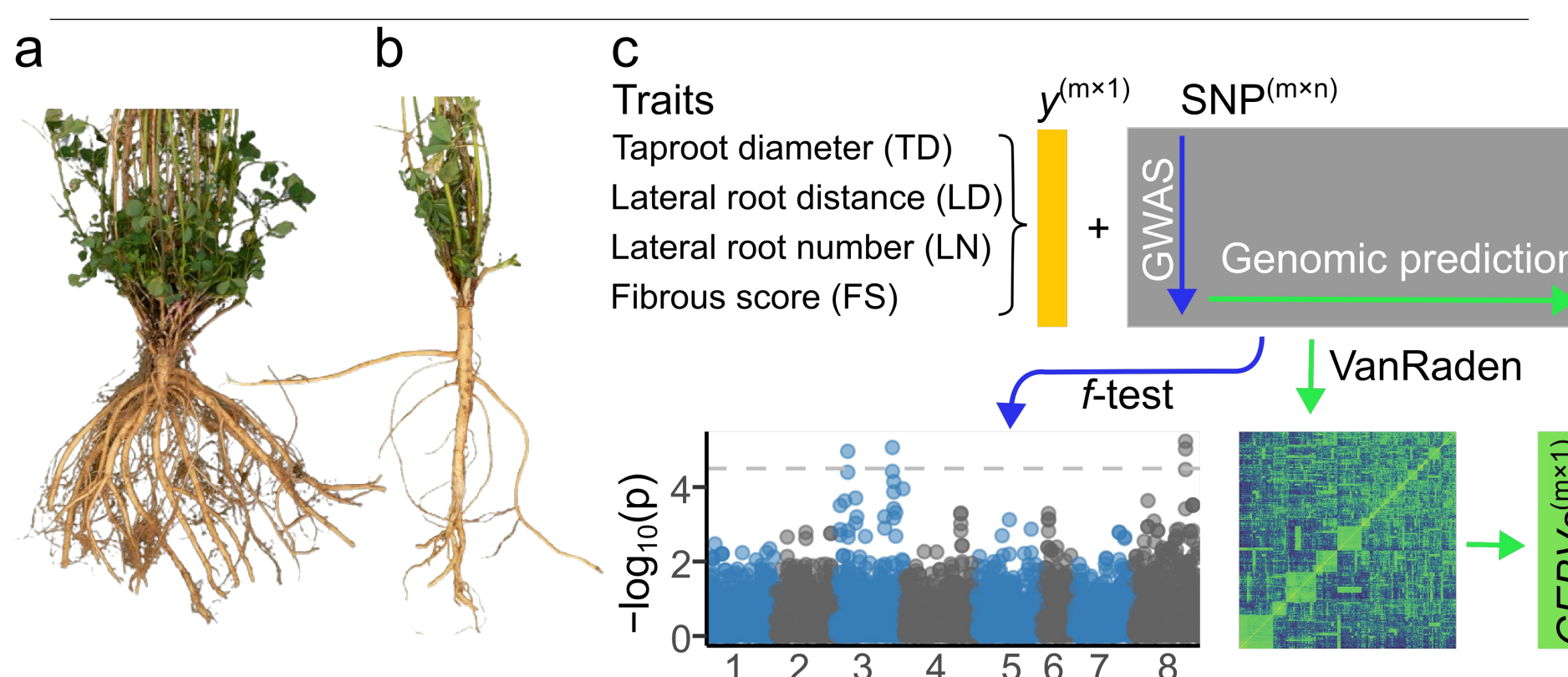| Cycle	RSA	Population	P&G	Geno	Total
0	0	UMN_2892	140	182	322
2	LF-T	UMN_2963	133	181	314
2	HF-B	UMN_2966	146	177	323
3	LF-T	UMN_3233	182	140	322
3	HF-B	UMN_3234	187	134	321
4	LF-T	UMN_4563	213	109	322
4	HF-B	UMN_4561	187	136	323
		Total	1,188	1,059	2,247

Figure 1. Flowchart of data analysis. Alfalfa root system architecture (RSA) was classified into a. High fibrous & branched root (HF-B) and **b.** Low fibrous & taproot (LF-T). **c.** GWAS and genomic prediction were applied to four phenotypic traits. y, vector of phenotypic response; SNP, marker matrix; VanRanden, genomic relationship matrix (VanRanden, 2007); GEVBs, genomic estimated breeding values.
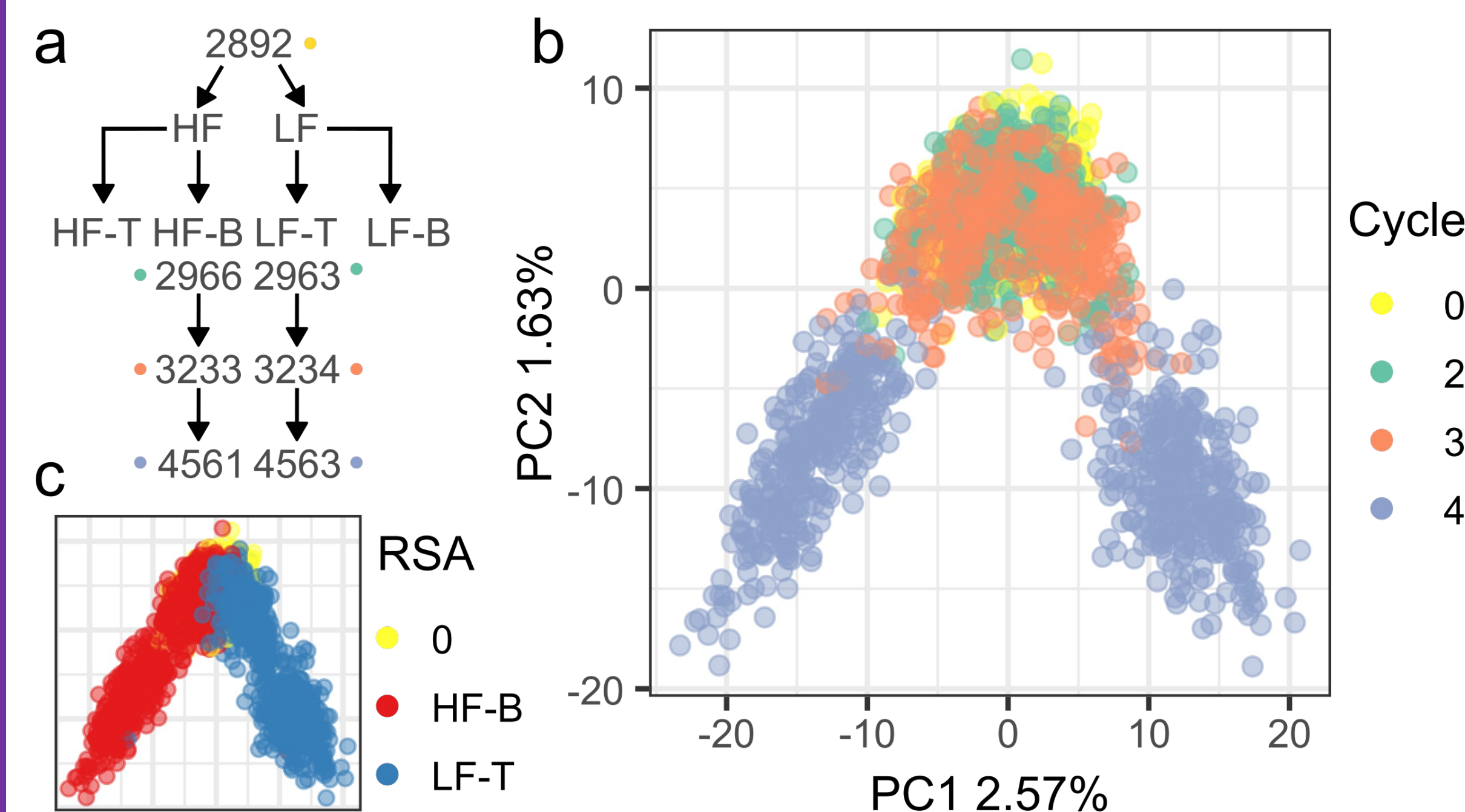
Results

Figure 2. Populations genotyped and principal component analysis (PCA) of RSA populations. a. Diagram of population generation over four divergent selection cycles. **b.** PCA of genotypes colored by selection cycle. **c.** PCA of genotypes colored by RSA. HF-B, high fibrous-branched root; LF-T, low fibrous-tap root.
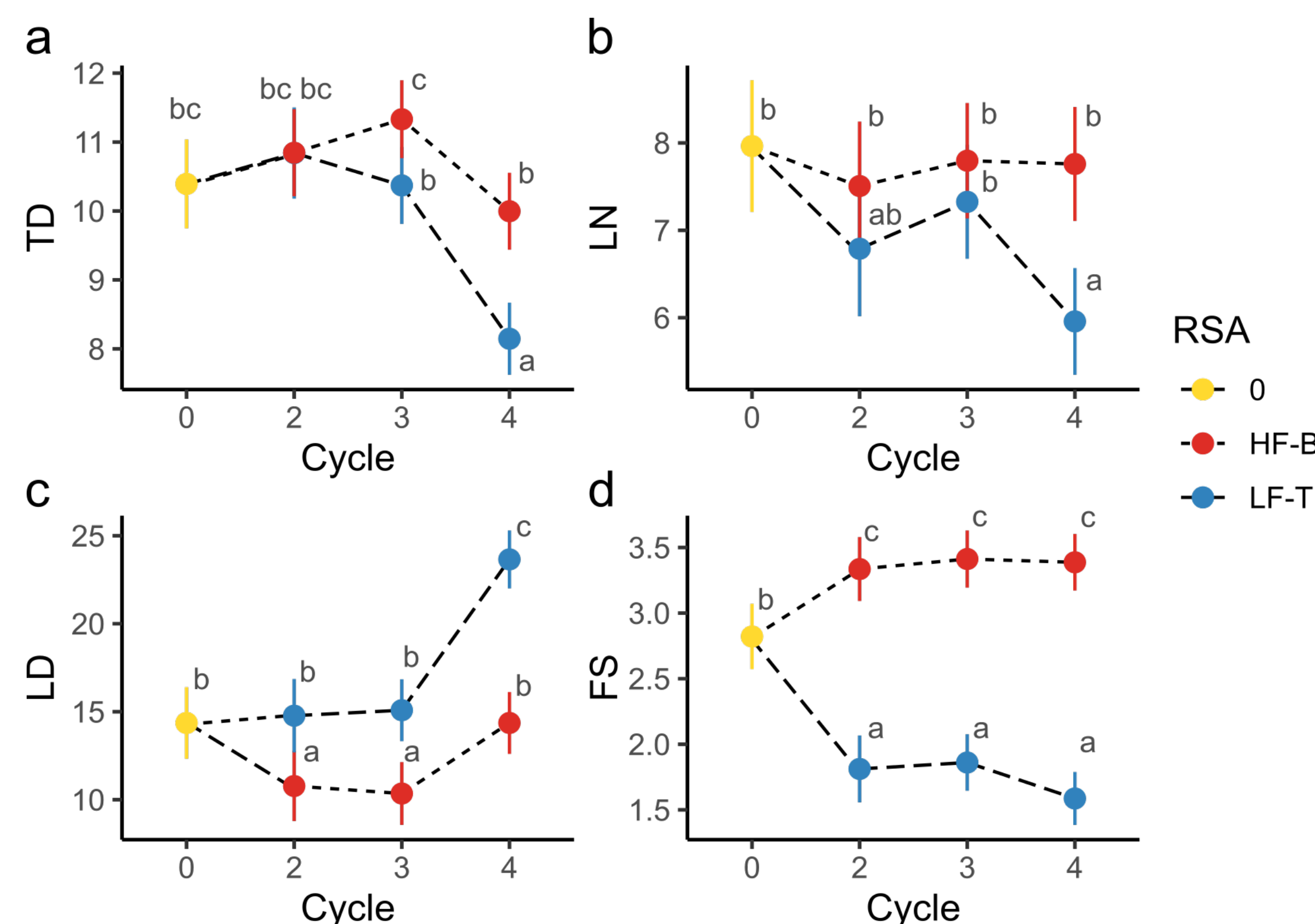

Figure 3. Least-squares means for four traits associated with RSA: a. Taproot diameter (TD) in mm. **b.** Lateral root number (LN). **c.** Lateral root distance (LD) in mm. **d.** Fibrous score (FS) (1-5). Different letters stand for significantly different means (p-value < 0.05) using Tukey's method for pairwise. Zero (0), parental population; HF-B, high fibrous-branched root populations; LF-T, low fibrous-tap root populations.

Table 2. Candidate markers and genes associated with different root traits. TD, LN, LD, and FS are described in Figure 3. Log(p) is the $-\log_{10}$ of p-values. R^2 corresponds to the phenotypic variance explained.

Trait	log(p)	Marker	R^2	Gene
TD	5.37	chr5.1_45250257	5.40E-03	–
TD	5.23	chr6.1_5216583	–	*RRM*
LN	4.96	chr3.1_32189111	1.49E-02	*JASON*
LN	5.06	chr3.1_81395244	–	
LN	5.34	chr5.1_4061189	3.17E-04	*ATPase*
LN	5.56	chr8.1_72832610	2.15E-03	*Pectate lyase*
LD	9.71	chr1.1_75061265	1.24E-01	*SPT13*
LD	4.8	chr2.1_24528970	–	*CRK*
LD	4.81	chr3.1_41090026	1.32E-02	*ATP-binding*
LD	4.65	chr3.1_69669859	2.50E-02	*LMBR1*
LD	4.76	chr5.1_616294	–	*START*
LD	5.06	chr5.1_6234907	–	*TIFY*
LD	5.08	chr6.1_52266116	7.42E-03	*PARP*
FS	4.91	chr1.1_26865008	8.43E-04	–

Table 3. Predicted ability (PA), genomic heritability (G.H2), and accuracy (ACC) of genomic prediction by GBLUP. TD, LN, LD, and FS are described in Figure 3.

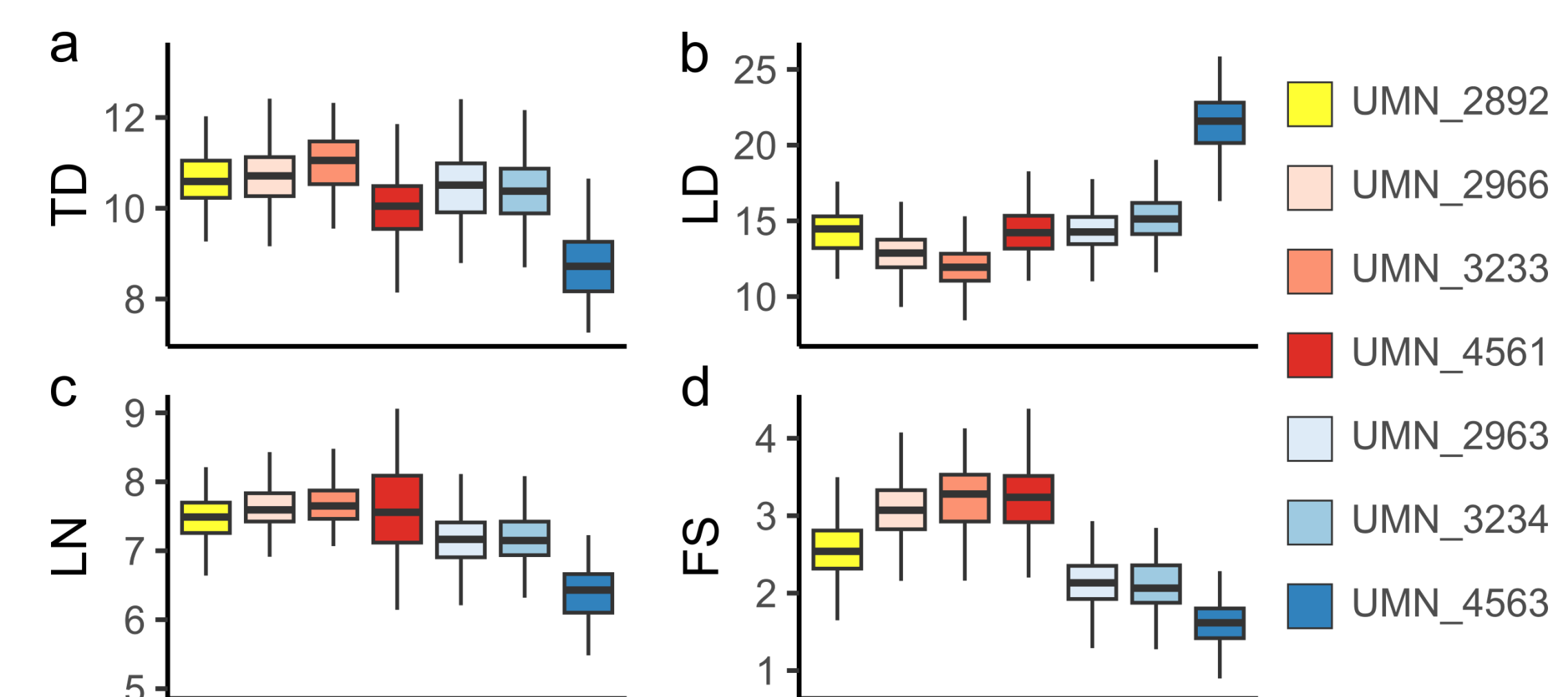| Source	TD	LN	LD	FS
PA	0.36	0.16	0.37	0.46
G.H^2	0.31	0.13	0.19	0.30
ACC	0.64	0.45	0.85	0.83

Figure 4. Boxplot of genomic estimated breeding values of testing population (1,059 genotypes). a. Taproot diameter (TD) in mm. **b.** Lateral root distance (LD) in mm. **c.** Lateral root number (LN). **d.** Fibrous score (FS) (1-5).

Conclusions

- GWAS identified potential markers and candidate genes associated with root development, while genomic prediction facilitated the selection of individual plants with high merit based on RSA-associated traits.
- GBLUP was successful for genomic selection and relative prediction accuracy. LN exhibited a medium prediction accuracy (0.45), while TD, FS, and LD had high prediction accuracy (0.64, 0.83, and 0.85, respectively).
- Implementing GEBVs in the genomic selection program will enable the alfalfa breeding community to improve the selection of individuals with high merit, developing drought-tolerant and more productive alfalfa cultivars.

References
1. Lynch, J. P. (2022). Harnessing root architecture to address global challenges. *Plant Journal* 109, 415–431. doi: 10.1111/tpj.15560.
2. Bucciarelli, B., Xu, Z., Ao, S., Cao, Y., Monteros, M. J., Topp, C. N., et al. (2021). Phenotyping seedlings for selection of root system architecture in alfalfa (Medicago sativa L.). *Plant Methods* 17.
3. Vanraden, P. M. (2007). Genomic measures of relationship and inbreeding. *Interbull Bulletin* 25, 33–33.