

Supplementary Materials

Table S1. Primers used for ISSR and SCAR analysis. The primer name, 5’-3’sequence and their respective annealing temperatures. ISSR-PCR conditions: 3 min at 94 °C, for initial denaturation, 36 cycles of 45 s at 94 °C for denaturation, 1 min at annealing temperature, 1 min at 72 °C for extension, followed by 5 min at 72 °C for a final extension of the single strands.

SCAR– PCR conditions: 94 °C for 3 min, 40 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by one last extension step of 5 min at 72 °C. ISSR amplified products were resolved on a 1.5% agarose gel while SCAR on a 2.5% agarose gel along with a 100–1000-bp ladder (ApplyChem GmbH, Darmstadt, Germany).

	Primer name	Nucleotide sequence 5' -> 3'	Annealing temperature
ISSR	Phv 06	CCA(CT) ₈	37 °C
	Phv 07	GTG(GT) ₈	38 °C
	Lol 2	(CT) ₈ GC	37 °C
	Lol 7	(GAGA) ₃ CC	34 °C
	Lol 8	(GT) ₆ CC	34 °C
	Lol 9	(CAC) ₃ GC	34 °C
	Lol 10	(GAG) ₃ GC	34 °C
	Lol 12	(GTG) ₃ GC	34 °C
SCAR	Phs F	AGCATATTCTAGAGGCCTCC	55 °C
	Phs R	GCTCAGTTCCTCAATCTGTTC	

Table S2. Seed morpho-colorimetric descriptors and analysis. Seed morpho-colorimetric parameters measured for each common bean population. Data represent the mean ($n=10$) \pm standard error. CV: Ciliegino; SMR: San Michele Rosso; FDA: Fagiolo d’Acqua.

Population	CV	SMR	FDA
Source	Vastogirardi (Molise) 41° 46' 9.651" (N) 14° 15' 28.894" (E)	Sarconi (Basilicata) 40° 14' 38.04" (N) 15° 51' 55.439" (E)	Pietrabbondante (Molise) 41° 43' 9.829" (N) 14° 22' 47.892" (E)
100 seed weight (g)	53.03 \pm 1.04	31.07 \pm 1.67	76.10 \pm 0.27
100 seed volume (mL)	80.33 \pm 0.33	50.667 \pm 0.67	101.33 \pm 0.03
100 seed density (g·mL ⁻¹)	0.66 \pm 0.01	0.614 \pm 0.04	0.75 \pm 0.03
Area (cm ²)	0.96 \pm 0.03	0.62 \pm 0.02	1.17 \pm 0.03
Perimeter (cm)	3.64 \pm 0.06	2.88 \pm 0.05	3.98 \pm 0.05
Major axis lenght (cm)	1.26 \pm 0.02	1.01 \pm 0.03	1.39 \pm 0.02
Minor axis lenght (cm)	0.97 \pm 0.02	0.78 \pm 0.01	1.07 \pm 0.02
Roundness	0.77 \pm 0.01	0.78 \pm 0.02	0.77 \pm 0.02
Min gray value	0.00 \pm 0.00	0.20 \pm 0.13	8.00 \pm 2.80
Max gray value	220.00 \pm 5.26	204.4 \pm 5.01	190.70 \pm 2.60
Mean gray value	18.42 \pm 0.73	19.747 \pm 1.27	89.59 \pm 2.75
Median gray value	7.20 \pm 0.44	8.70 \pm 1.12	92.40 \pm 3.74
Modal gray value	2.70 \pm 0.62	4.50 \pm 1.22	106.40 \pm 6.00
Standard deviation gray value	24.74 \pm 0.68	29.16 \pm 1.34	32.87 \pm 1.65



Ciliegino – CV



San Michele Rosso – SMR



Fagiolo d'Acqua – FDA

Figure S1. Plant material collection. Seeds of three Italian common bean (*Phaseolus vulgaris* L.) landrace populations cultivated in Italian hilly and mountainous areas and stored in Molise Germplasm Bank. Ciliegino and Fagiolo d'Acqua are cultivated in Molise marginalized territories, Vastogirardi and Pietrabbondante, respectively, both belonging to Alto Medio Sannio Inner Area. San Michele Rosso is cultivated in Sarconi (PZ), Basilicata region.

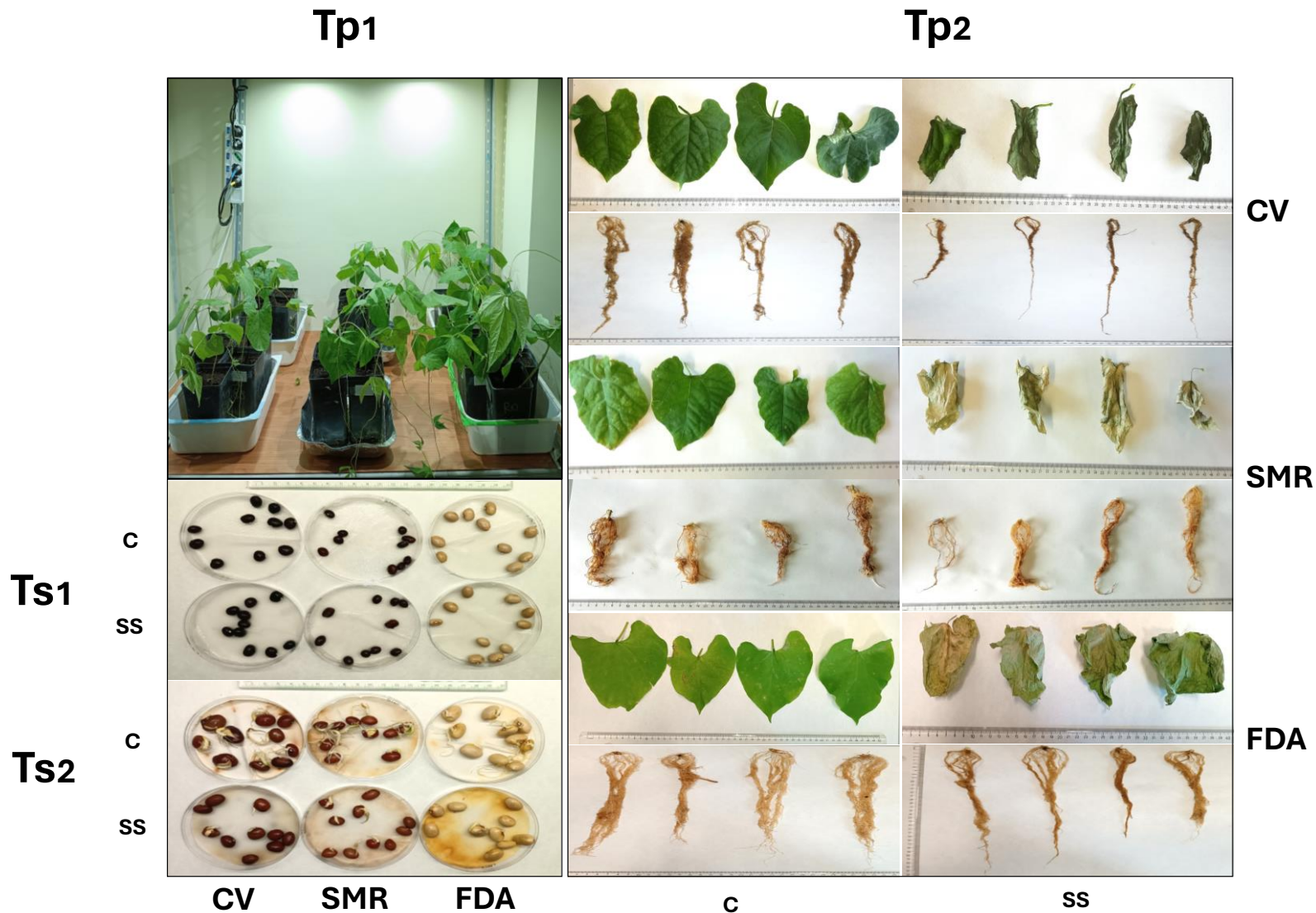


Figure S2. Common bean plants and seeds subjected to salt treatment. Common bean plants before the treatment (Tp₁); common bean leaves and roots collected after 15 days of growth under control and salt stress (Tp₂); seeds at the beginning (Ts₁) and at the end (Ts₂) of germination test. One replicate for each treatment. C: control; SS: salinity stress (NaCl 200 mM); CV: Ciliegino; SMR: San Michele Rosso; FDA: Fagiolo d'Acqua.

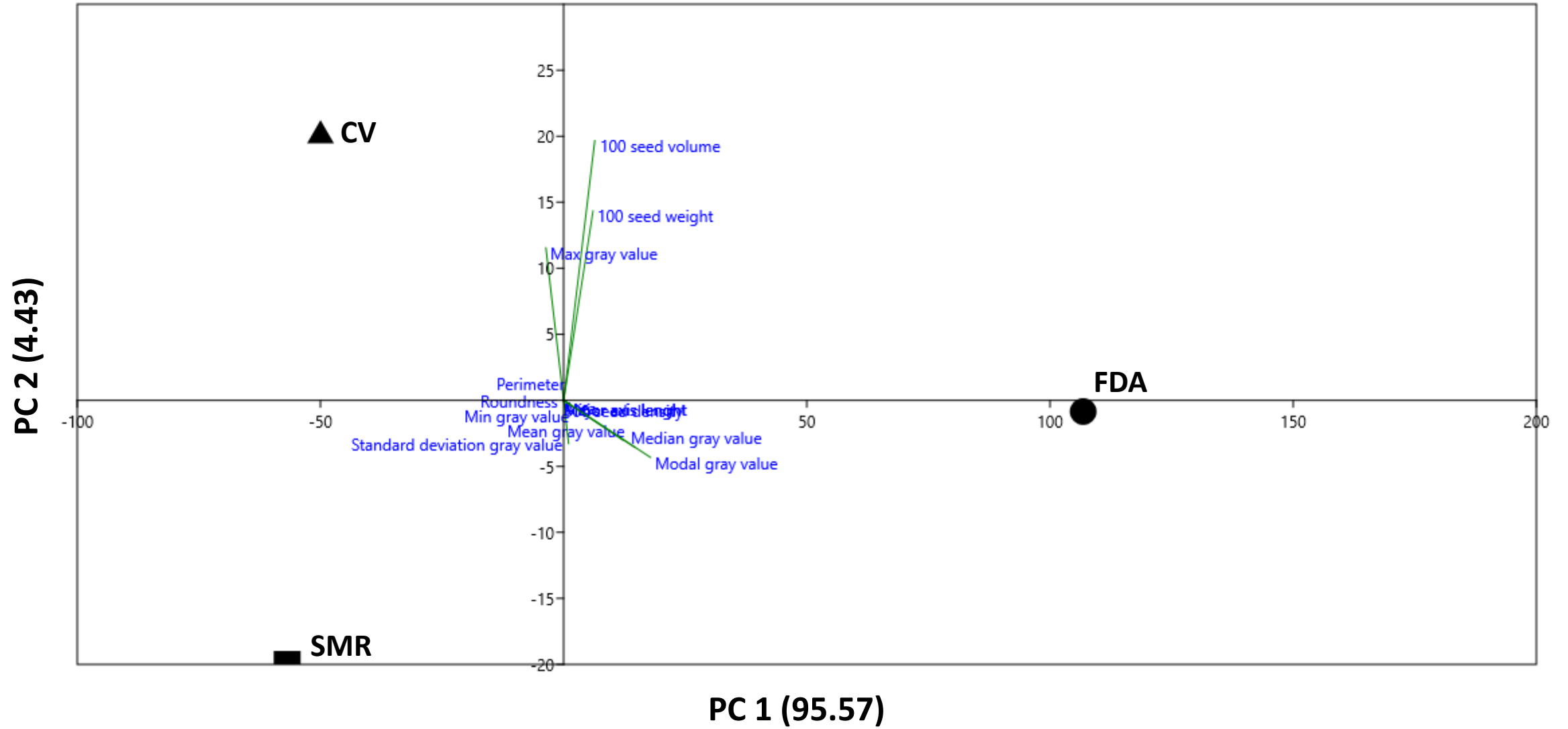
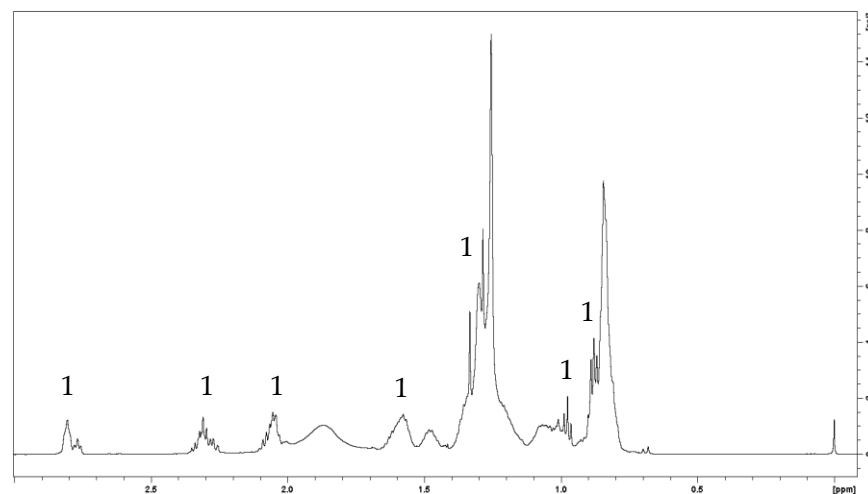


Figure S3: Most discriminant variables in seed morpho-colorimetric analysis. Seed volume, seed weight, max gray value, median gray value and modal gray value resulted to be the most discriminative variables in differentiating the common bean populations. CV: Ciliegino; SMR: San Michele Rosso; FDA: Fagiolo d'Acqua.

a

Metabolite	Assignment	δ ^1H	
Fatty acids	1	ω_1 - CH_3	0.86 t
		ω_3 - CH_3	0.97 (t, 8)
		$-(\text{CH}_2)_n$ -	1.28 m
		γ - CH_2	1.61m
		Allylic hydrogen	2.06 m
		β - CH_2	2.33 m
		Bis-allylic hydrogen	2.81 m
Glycerol	2	CH_2 -1,3	4.22 dd
PUFA	3	$\text{CH}=\text{CH}$	5.37 m

b



c

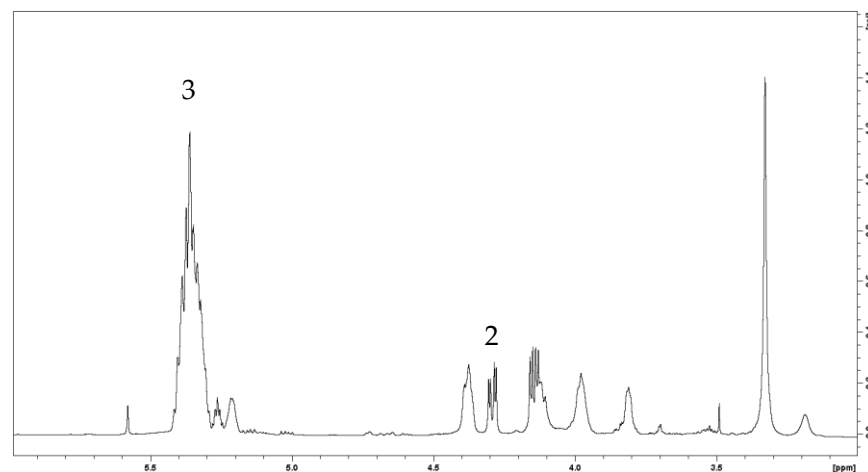


Figure S4. ^1H NMR metabolomic profile of common bean seed lipophilic extracts. ^1H chemical shift assignment (δ , ppm) of the metabolites detected in seed lipophilic extracts of common bean populations (a). Expanded ^1H NMR spectrum from 0.0 to 3.0 ppm (b) and from 3.0 to 6.0 ppm (c). d: doublet, dd: double doublet; m: multiplet; s: singlet, t: triplet; tp: triple doublet.

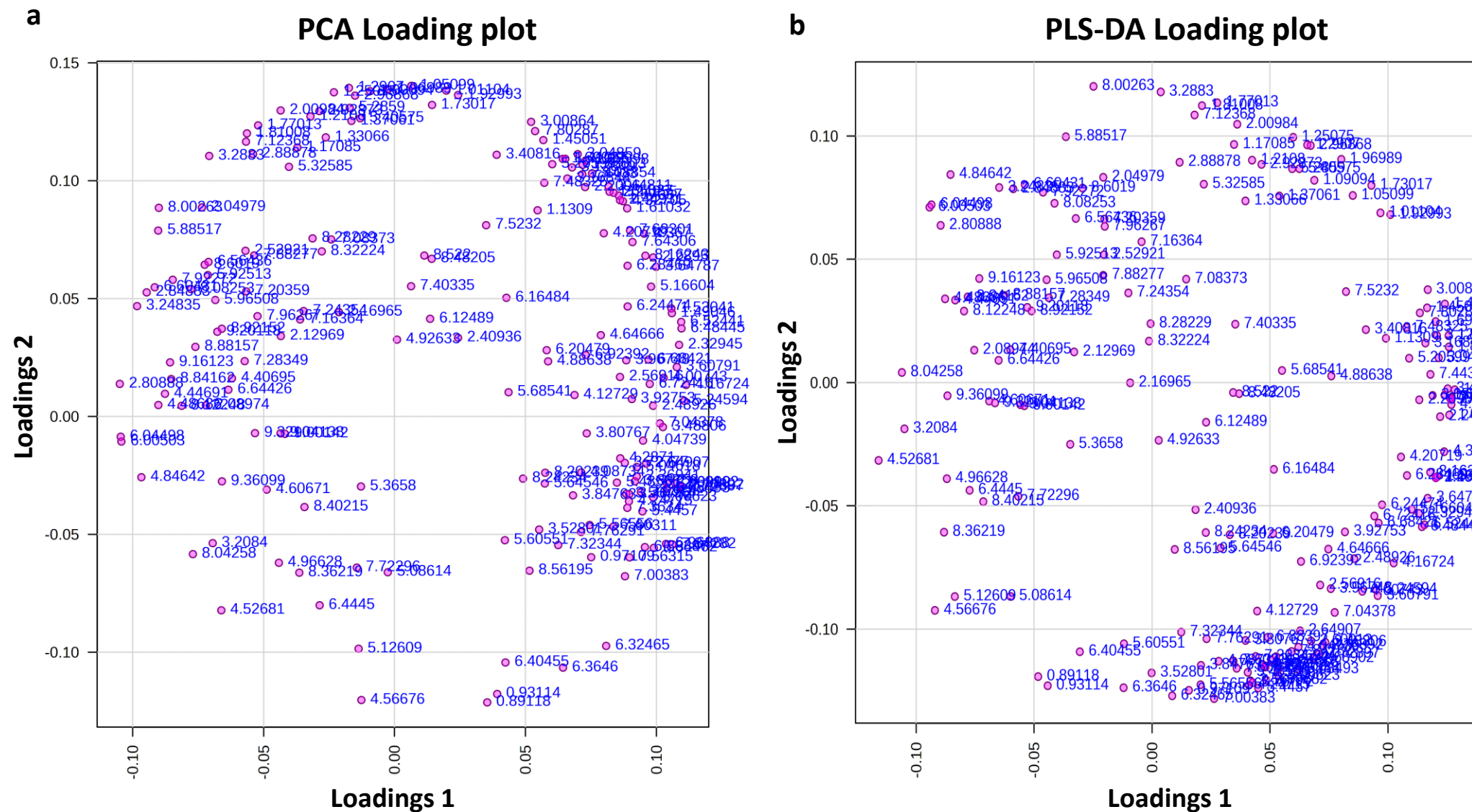


Figure S5. Loadings plot of the corresponding Principal Component Analysis (PCA) and Partial-Least-Squares Discriminant-Analysis (PLS-DA) computed on NMR qualitative data of common bean aqueous seed extracts. Loading plot of the PCA (a) and the loading plot of the PLS-DA (b). The numbers reported refer to the chemical shifts.

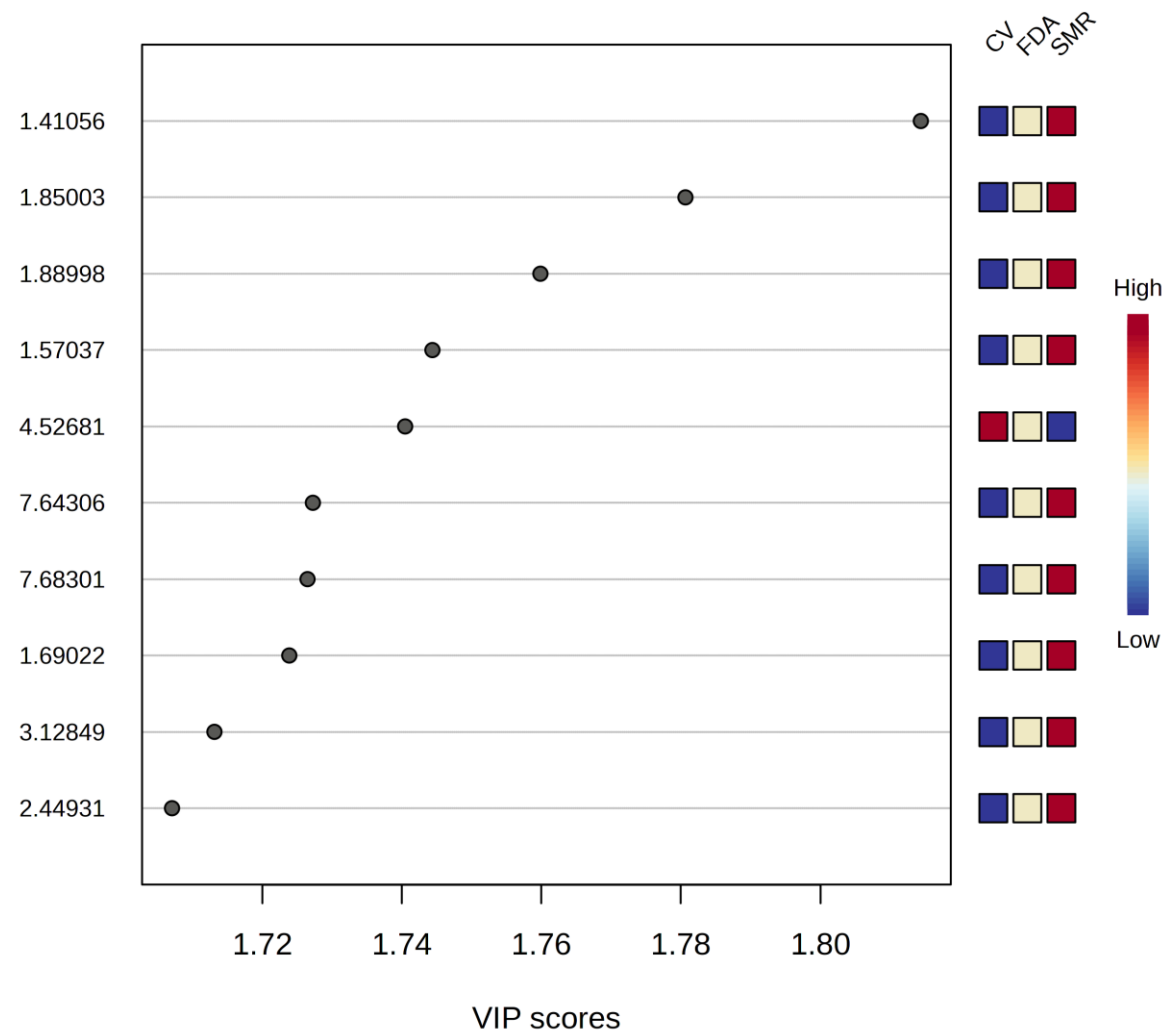


Figure S6. Variables Importance in Projection (VIPs) plot of PLS-DA. VIPs plot representing the 10 most discriminant metabolites in common bean populations. The x-axis reports VIPs scores, the y-axis shows the chemical shifts (in ppm) which can be assigned to corresponding metabolites by referring to Figure 2a. The coloured boxes on the right indicate the relative concentrations (red, high; yellow, intermediate; blue, low) of the corresponding metabolite in each bean population under study . CV: Ciliegino; SMR: San Michele Rosso; FDA: Fagiolo d'Acqua.