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Using SCoT and SRAP Markers to Assess Genetic Diversity in Wild and Cultivated *Hordeum* Genotypes

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ABSTRACT

Understanding how cultivated barley relates to its wild relatives is important if we want to reduce genetic erosion and keep broad, useful diversity in breeding programs. Among wild *Hordeum* species, *Hordeum bulbosum* is especially relevant because it is cross-compatible with cultivated barley (*Hordeum vulgare*) and can provide adaptive variation for pre-breeding. In this study, we analyzed genetic relationships in 50 *Hordeum* genotypes (44 *H. bulbosum* and 6 cultivated *H. vulgare*) using two dominant marker systems, SCoT (Start Codon Targeted) and SRAP (Sequence-Related Amplified Polymorphism). Amplification profiles were clear and reproducible and only unambiguous bands were retained for binary scoring. Across both systems, 160 loci were scored, of which 138 were polymorphic. SCoT generated 79 loci, including 64 polymorphic loci; polymorphism per primer ranged from 75% to 100%, with PIC values between 0.12 and 0.26. SRAP generated 81 loci, including 77 polymorphic loci; polymorphism per combination ranged from 85.7% to 100%, with PIC values between 0.22 and 0.33. UPGMA clustering of the combined matrix clearly separated cultivated controls from wild accessions and also identified a distinct Hb4X subgroup within *H. bulbosum*. PCA supported the same structure and the high cophenetic correlation confirmed strong agreement between the dendrogram and the original dissimilarity matrix. Overall, the combined SCoT and SRAP approach provided a robust and practical framework for germplasm structuring and parent selection in barley pre-breeding.

Keywords: *Hordeum bulbosum*, *Hordeum vulgare*, genetic diversity, SCoT, SRAP.

INTRODUCTION

Over recent decades, breeding programs have delivered substantial gains in crop yield and productivity. However, these gains have often relied on repeated use of a relatively narrow set of elite lines, which can reduce the diversity available for adaptation to biotic and abiotic stresses. At the same time, agriculture is increasingly affected by climate change, population growth, and pressure on arable land, reinforcing the need for more resilient and adaptable germplasm. Under these conditions, broadening the genetic base of crops has become a major breeding priority. Crop wild relatives are therefore receiving renewed attention as sources of allelic diversity and adaptive traits that can help counter genetic erosion and improve resilience (Zhang and Batley, 2020; El Haddad et al., 2021; Khoury et al., 2022).

Barley (*Hordeum vulgare* L.; *Poaceae*) is one of the major cereal crops worldwide and has been cultivated since early agriculture. Archaeological and genetic evidence indicates that domestication occurred in the Fertile Crescent around 10,000 years ago, from its wild progenitor, *H. vulgare* ssp. *spontaneum* (Badr et al., 2000; Saisho and Purugganan, 2007; Zohary et al., 2012). Within this broader context, the genus *Hordeum* includes numerous taxa with different cytotypes (diploid, tetraploid and hexaploid) and many species are perennial (Bothmer et al., 1995). Together, this taxonomic and cytogenetic diversity expands the pool of potentially useful variation beyond what is currently captured in modern cultivated barley.

Among barley wild relatives, *Hordeum bulbosum* is particularly relevant because it is a close perennial relative of cultivated barley and a representative of barley's secondary gene pool. It is cross-compatible with *H. vulgare* and has been used in pre-breeding and introgression efforts aimed at broadening

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the cultivated gene pool. Its breeding value is supported by reports describing transfer of resistance to powdery mildew, leaf rust, stem rust, scald and viral diseases, together with improvements in traits linked to adaptation and productivity (Pickering et al., 1995; Walther et al., 2000; Ruge et al., 2003; Singh et al., 2004; Shtaya et al., 2007; Fetch Jr. et al., 2009; Scholz et al., 2009; Johnston et al., 2013; Hoseinzadeh et al., 2020; Yu et al., 2022; Pidon et al., 2024). In parallel, molecular approaches have facilitated the detection and characterization of introgressed *H. bulbosum* chromatin in barley backgrounds, enabling more targeted use of this resource (Wendler et al., 2014).

Because *H. bulbosum* is a perennial wild relative, it is also relevant to current discussions on the role of perennial germplasm in cereal improvement under changing environments (Westerbergh et al., 2018; Chapman et al., 2022). More broadly, perennial and regrowth-capable germplasm is increasingly considered a source of traits associated with persistence and multi-season stability, even though cultivated barley itself is an annual crop (Hayes et al., 2018; Chapman et al., 2022). For this reason, diversity studies in *H. bulbosum* can inform both near-term pre-breeding and longer-term strategies aimed at broadening functional diversity in barley.

A clear understanding of genetic relationships within and between cultivated and wild *Hordeum* germplasm is essential for germplasm management and parental selection. In practice, molecular markers are widely used to characterize diversity and complement phenotypic evaluations. In barley, marker systems are selected according to the study objective and available resources, ranging from RAPD and ISSR for rapid screening (Serpoush et al., 2022), SSRs for diversity and structure analyses (Dido et al., 2022), to SNP platforms for higher-throughput genome-wide characterization (Elakhdar et al., 2018; Almerekova et al., 2021; Yirgu et al., 2023).

In addition to SSR and SNP technologies, gene-targeted PCR-based marker systems remain attractive in many laboratories because they are relatively simple, cost-effective, and informative without requiring prior sequence information. Two such systems are SCoT (Start Codon Targeted) and SRAP (Sequence-Related Amplified Polymorphism).

SCoT markers, introduced by Collard and Mackill (2009), target conserved regions flanking the ATG start codon and generate multilocus profiles using a single primer. SRAP markers, developed by Li and Quiros (2001), preferentially amplify open reading frames (ORFs) using a two-primer system and have been widely used for diversity assessment and genotype discrimination. Evidence for the usefulness of SCoT and SRAP markers is available across a wide range of plant taxa. SCoT has been applied for diversity and relationship analyses in wheat and other crops (Ma et al., 2022; Shaban et al., 2022; Abouseada et al., 2023; Hromadová et al., 2023). Similarly, SRAP has been used for diversity studies and relationship inference in cereals and other species (Khaled et al., 2021; Dabral et al., 2022; Almarri et al., 2023; Galal et al., 2023; Mohammed et al., 2023; Zagorcheva et al., 2024).

In barley specifically, SCoT markers have been used to assess diversity and relationships among accessions, either alone or in combination with other marker systems (Güngör et al., 2022; Ghonaim et al., 2023; Tahir et al., 2023). SRAP has also been applied to characterize genetic diversity in barley germplasm, including materials evaluated under stress- and resistance-related contexts (Abaza et al., 2022; Elshafei et al., 2024). Taken together, these studies indicate that SCoT and SRAP are practical tools for diversity assessment in *Hordeum* and can provide complementary information.

Comparative assessments that include both wild and cultivated *Hordeum* remain useful, because marker-based diversity patterns can vary with the origin and representation of the analyzed germplasm. This type of characterization can support both germplasm management and pre-breeding decisions. Therefore, the objective of this study was to assess genetic relationships among 50 *Hordeum* accessions (44 *H. bulbosum* and six *H. vulgare*) using SCoT

and SRAP markers and to describe patterns of diversity within and between wild and cultivated genotypes.

MATERIAL AND METHODS

Plant material - a total of 50 Hordeum accessions from the NARDI Fundulea collection were analyzed, including 44 wild genotypes of *H. bulbosum* and six cultivated genotypes of *H. vulgare*. Genotype codes and group assignments are provided in Table S1.

Genomic DNA extraction and quantification - genomic DNA was extracted from fresh leaf tissue using the NucleoSpin Plant II kit (Macherey-Nagel GmbH & Co) following the manufacturer's instructions. DNA quality was checked on 0.8% agarose gels. DNA concentration was measured by fluorescence using a FLUOstar Omega microplate reader, and samples were subsequently diluted to 30 ng µL prior to PCR.

SCoT marker analysis - Amplifications were performed using eight primers (SCoT2, SCoT9, SCoT12, SCoT13, SCoT16, SCoT20, SCoT24 and SCoT28); primer sequences are provided in Table 1. PCR reactions were carried out in 20 µL containing 30 ng template DNA, 1× DreamTaq Green PCR Master Mix (Thermo Scientific) and 0.6 µM primer. Amplifications were performed on a ProFlex thermocycler (Applied Biosystems) using the following profile: 95°C for 3 min; 38 cycles of 95°C for 45 s, 48°C for 1 min and 72°C for 2 min and a final extension at 72°C for 10 min.

SRAP marker analysis - was performed using six primer combinations (SRAP8, SRAP20, SRAP59, SRAP75, SRAP80 and SRAP85). Forward and reverse primer sequences are provided in Table 1. PCR reactions were carried out in 20 µL containing 30 ng template DNA, 1× DreamTaq Green PCR Master Mix (Thermo Scientific) and 0.6 µM of each primer (forward and reverse) per reaction. Amplification was performed on.

Table 1. SCoT and SRAP primers and sequences

No	Marker	Primer sequence	No	Marker	Primer sequence
1	**SCoT2**	CAACAATGGCTACCACCC	1	**SRAP8**	F: TGAGTCCAAACCGGTGC R: GACTGCGTACGAATTCAA
2	**SCoT9**	CAACAATGGCTACCAGCA	2	**SRAP20**	F: TGAGTCCAAACCGGTCC R: GACTGCGTACGAATTTGA
3	**SCoT12**	ACGACATGGCGACCAACG	3	**SRAP59**	F: TGAGTCCAAACCGGTGC R: GACTGCGTACGAATTAAT
4	**SCoT13**	ACGACATGGCGACCATCG	4	**SRAP75**	F: TGAGTCCAAACCGGACC R: GACTGCGTACGAATTCAG
5	**SCoT16**	ACCATGGCTACCACCGAC	5	**SRAP80**	F: TGAGTCCAAACCGGACC R: GACTGCGTACGAATTTGC
6	**SCoT20**	ACCATGGCTACCACCGCG	6	**SRAP85**	F: TGAGTCCAAACCGGAAG R: GACTGCGTACGAATTCTG
7	**SCoT24**	CACCATGGCTACCACCAT			
8	**SCoT28**	CCATGGCTACCACCGCCA			

Electrophoresis and visualization - PCR products were separated on 1.5% agarose gels stained with ethidium bromide. Fragment sizes were estimated using a 100 bp DNA ladder (Cleaver Scientific). Gels were visualized and documented using a UVITEC HD6 imaging system.

Band scoring and downstream analyses - only clear and reproducible bands were scored. Band presence was coded as 1 and absence as 0 to generate a binary matrix for each marker system. In addition, selected samples were re-scored as technical checks during band annotation; duplicate entries were not treated as independent genotypes in downstream analyses.

Data analysis - binary matrices were imported into BIO-R for diversity analysis. Rogers' genetic distance was used to compute pairwise dissimilarities, followed by

UPGMA clustering. PCA was performed on the combined matrix. Dendrogram fidelity was evaluated using the cophenetic correlation coefficient.

RESULTS AND DISCUSSION

Representative gel profiles and band scoring - SCoT and SRAP assays produced clear and reproducible amplification profiles across the 50 analyzed *Hordeum* genotypes. To illustrate band quality and polymorphism, one representative SCoT molecular marker (SCoT13; Figure 1) and one representative SRAP molecular marker (SRAP20; Figure 2) were selected for the main text. These profiles show both shared bands (present across multiple genotypes) and differential bands (present only in subsets of genotypes), supporting their suitability for diversity analysis. For matrix construction, only unambiguous and reproducible fragments were retained, whereas faint or unclear bands were excluded. Bands were scored in binary format as presence (1) or absence (0) and the resulting matrices were used for all downstream analyses. A small subset of samples was re-scored as a technical check during annotation; duplicate entries were not treated as independent genotypes in the final dataset.

Marker performance and polymorphism - both marker systems were informative in this panel. In total, 160 loci were scored, of which 138 were polymorphic, corresponding to 86.25% polymorphism at combined-dataset level (Table 2). For the SCoT system, eight primers generated 79 scorable loci, including 64 polymorphic loci (81.0%). Polymorphism per primer ranged from 75.0% to 100%. The highest polymorphism rate was observed for SCoT20 and SCoT24 (100%), and these two primers also produced the highest number of total and polymorphic loci (12 and 12, respectively). Primer-level PIC values ranged from 0.12 to 0.26 (mean 0.19).

For SRAP system, six primer combinations generated 81 scorable loci, of which 77 were polymorphic (95.1%). Polymorphism per combination ranged from 85.7% to 100%. The highest polymorphism (100%) was recorded for SRAP8, SRAP20, and SRAP59. Among SRAP combinations, SRAP20 yielded the largest number of total loci (19) and polymorphic loci (19). PIC values ranged from 0.22 to 0.33 (mean 0.27).

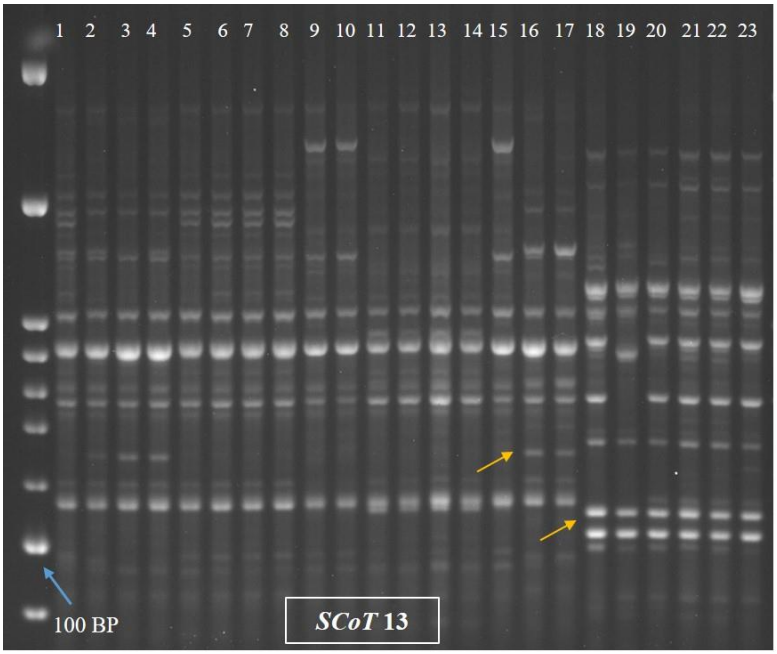

Figure 1. Representative SCoT amplification profile (SCoT13) across *Hordeum* genotypes.
PCR fragments were resolved by agarose gel electrophoresis.
The profile illustrates clear and polymorphic banding suitable for binary scoring.

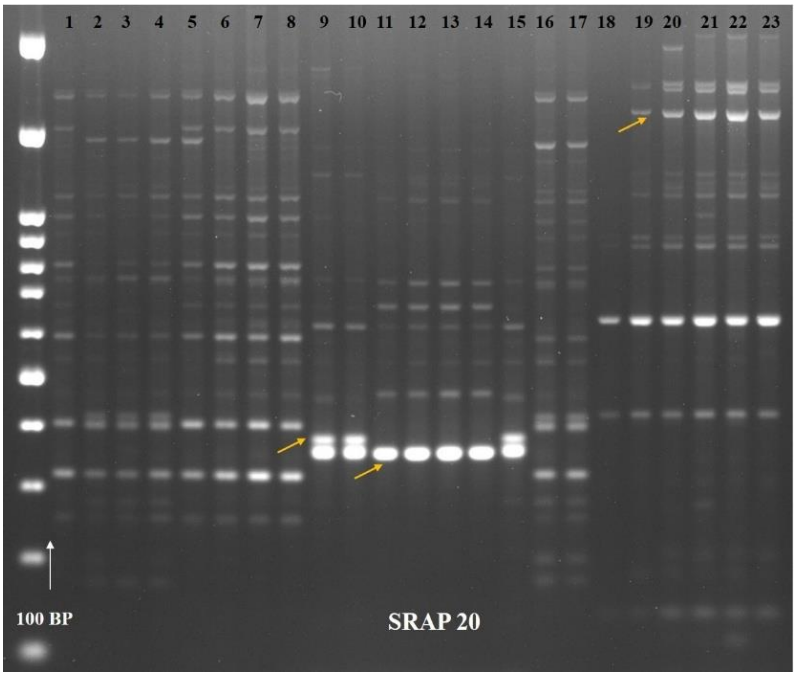

Figure 2. Representative SRAP amplification profile (SRAP20) across *Hordeum* genotypes. The gel shows both shared and differential fragments among genotypes.

Overall, SRAP detected a higher proportion of polymorphic loci and slightly higher marker informativeness than SCoT in this panel. At the same time, both systems contributed complementary variation and the combined SCoT and SRAP matrix was therefore used as the primary dataset for clustering and ordination analyses. Based on this combined informative signal, the concatenated SCoT and SRAP matrix was used as the primary dataset for clustering and ordination analyses. Primer-level SCoT performance (including PIC and polymorphism percentages) and SRAP combination-level outputs are reported in the marker summary table.

Table 2. Per-marker summary of polymorphism and informativeness for SCoT and SRAP datasets

Marker	Scored loci (n)	Polymorphic loci (n)	Polymorphism (%)	PIC
SCoT2	8	6	75.0	0.18
SCoT9	9	8	88.9	0.23
SCoT12	8	6	75.0	0.14
SCoT13	11	10	90.9	0.22
SCoT16	12	12	100.0	0.26
SCoT20	9	8	88.9	0.15
SCoT24	12	12	100.0	0.12
SCoT28	10	9	90.0	0.25
Overall	79	64	81.0	0.19
SRAP8	12	12	100.0	0.29
SRAP20	19	19	100.0	0.33
SRAP59	15	15	100.0	0.26
SRAP75	13	12	92.3	0.26
SRAP80	15	13	86.7	0.24
SRAP85	7	6	85.7	0.22
Overall	81	77	95.1	0.27

Note: PIC for dominant markers was calculated as PIC = 2p(1−p), where p is the frequency of band presence.

Cluster analysis based on UPGMA dendrograms - genetic relationships among the 50 *Hordeum* genotypes were inferred from the combined SCoT and SRAP binary matrix using Rogers' genetic distances and UPGMA clustering (Figure 3). In the tree visualization, branches were color-coded to highlight the main biologically relevant groups (*H. vulgare* and wild *H. bulbosum*). The cophenetic correlation coefficient of the combined tree was very high (CP ≈ 0.990), indicating an excellent agreement between the dendrogram topology and the original distance matrix, and therefore a reliable hierarchical representation of pairwise genetic relationships.

The combined dendrogram resolved a clear and biologically coherent structure. The six cultivated *H. vulgare* (CC21-03, CC21-05, CC01-04, CC01-05, CC01-08, CC01-09) formed a compact and distinct cluster, clearly separated from the wild *H. bulbosum* fraction. This consistent separation supports the discriminatory capacity of the marker set at the interspecific level and confirms that the generated binary matrix captures robust divergence between cultivated barley and wild *Hordeum* material.

Within the wild material, Hb4X accessions formed a distinct subgroup, separated from most diploid *H. bulbosum* accessions. In contrast, Hb3 and Hb4 accessions were distributed across neighboring branches with partial overlap, rather than strict label-based partitioning. This indicates structured diversity within the diploid wild pool that is not fully captured by collection labels alone.

Importantly, the clustering pattern did not indicate random dispersion; instead, it showed hierarchical organization with both compact subclusters and gradual transitions among related wild genotypes. This is relevant for germplasm management, because it points to the coexistence of clearly differentiated groups (cultivated forms and Hb4X) and a more continuous diversity space within diploid *H. bulbosum*. In practical terms, this structure supports two complementary sampling strategies for downstream work: (i) selection of distant representatives from major branches to maximize diversity capture, and (ii) targeted sampling within tighter subclusters when near-related comparisons are needed.

When marker systems were analyzed separately (SCoT - Figure S1; SRAP - Figure S2), the major biological separations were broadly preserved, especially the distinct position of cultivated controls and the recognizable placement of Hb4X. However, branch arrangement within diploid wild accessions was somewhat marker-dependent, which is expected given differences in genomic targets and polymorphism profiles. The combined dataset provided the most stable and interpretable overall topology and was therefore used as the main framework for interpretation.

Taken together, UPGMA results indicate that both marker systems are informative, but their integration increases resolution and clustering robustness. The observed topology supports a clear interspecific split (*H. vulgare* vs *H. bulbosum*), a differentiated Hb4X subgroup within the wild pool and substantial but structured diversity among Hb3/Hb4 diploid accessions.

Principal Component Analysis (PCA) - based on the combined SCoT and SRAP binary matrix was consistent with the UPGMA clustering pattern (Figure 4). In the 2D projection, PC1 and PC2 explained 41.38% and 22.43% of the total variation, respectively, accounting together for 63.81%.

The scatterplot resolved three major groups: (i) the cultivated H. *vulgare* controls, (ii) the Hb4X subgroup, and (iii) a broader cluster including diploid H. *bulbosum* accessions (Hb3/Hb4). The cultivated controls formed a compact and clearly separated cloud, whereas Hb3/Hb4 genotypes showed wider dispersion, indicating higher within-group heterogeneity.

The 3D PCA supported the same structure (Figure S3). Inclusion of PC3 (8.74%) increased the cumulative variance explained by the first three axes to 72.55%, while preserving the major group configuration observed in 2D. Together, these results indicate that the combined marker set captures a stable and biologically meaningful

differentiation pattern across the analyzed panel.

Concordance between hierarchical clustering and dissimilarity structure - the cophenetic correlation coefficient of the combined UPGMA dendrogram was high (CP = 0.990), indicating an excellent fit between the dendrogram topology and the original pairwise dissimilarity matrix. This supports the robustness of the inferred group structure and strengthens confidence in downstream biological interpretation.

Biological interpretation of the observed pattern - the marker based dataset supports a structured pattern of genetic relationships in the analyzed panel and indicate both clear between group differentiation and substantial within group variation.

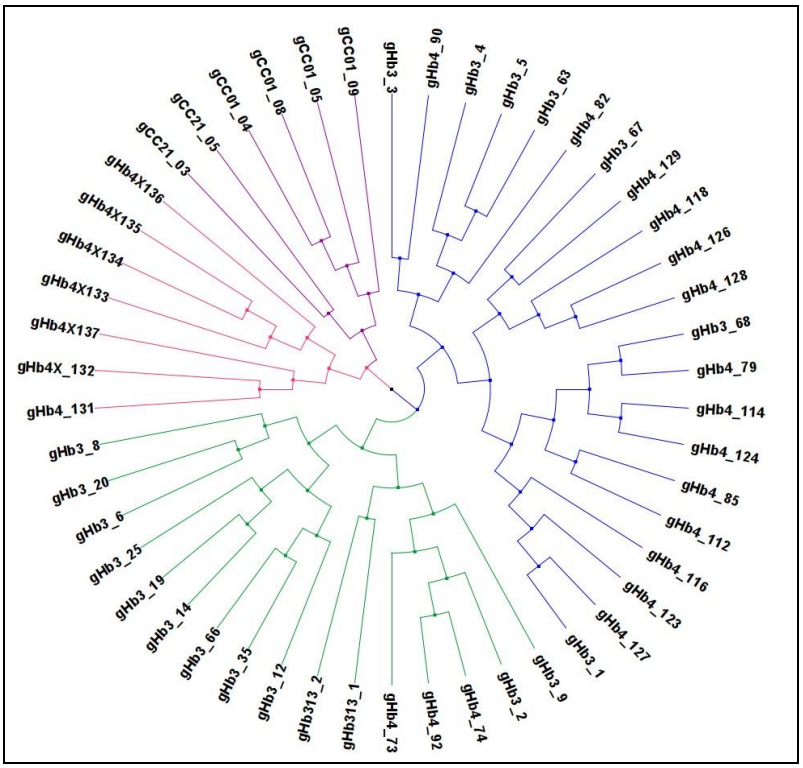

Figure 3. UPGMA dendrogram based on Rogers' genetic distance calculated from the combined SCoT and SRAP binary matrix for 50 *Hordeum* genotypes

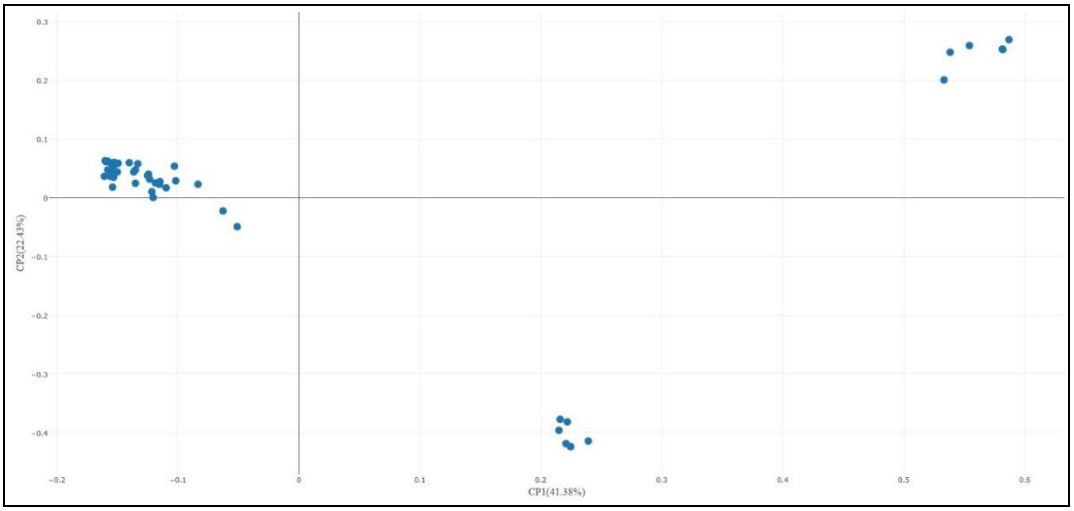

Figure 4. Principal Component Analysis plot of *Hordeum* genotypes based on the combined SCoT and SRAP binary matrix

Across methods, cultivated H. *vulgare* controls were consistently differentiated from wild H. *bulbosum* accessions, while Hb4X entries tended to form a recognizable subgroup. In contrast, diploid Hb3/Hb4 accessions showed broader internal spread and partial overlap, indicating heterogeneous diversity within the wild fraction.

These results should be interpreted as a robust description of molecular structure under the present marker set and sampling design. The observed grouping patterns provide a useful framework for germplasm organization and for subsequent selection steps, while finer biological inference should be further evaluated with additional datasets and trait-linked analyses.

The combined ScoT and SRAP framework proved effective for resolving genetic relationships in the analyzed *Hordeum* panel. The level of polymorphism obtained in the combined dataset shows that these marker systems captured substantial variation across both wild and cultivated materials. In this dataset, SRAP contributed a higher proportion of polymorphic loci than ScoT, while ScoT still provided useful discriminatory signal, confirming that the two systems are complementary rather than redundant. Our findings are consistent with other barley studies indicating that ScoT and SRAP markers can capture informative levels of polymorphism and support genotype discrimination (Habiba et al., 2021; Abaza et al., 2022; Güngör et al., 2022; Ghonaim et al., 2023; Tahir et al., 2023; Elshafei et al., 2024). Although marker performance is dataset-dependent, the higher polymorphism observed for SRAP in the present panel agrees with published evidence that different dominant systems may contribute unequally but complementarily to diversity inference. This supports the use of a combined ScoT and SRAP framework when a robust stratification of Hordeum germplasm is required.

A key result across analyses was the consistent separation of cultivated barley controls from wild *H. bulbosum* accessions. This pattern was recovered by both UPGMA clustering and PCA, indicating that the observed differentiation was not method-specific. The high cophenetic correlation further supports the reliability of the hierarchical structure inferred from the dissimilarity matrix.

Within the wild genotypes, diversity was structured but non-uniform. Hb4X accessions formed a coherent subgroup, while diploid Hb3/Hb4 accessions showed broader dispersion and partial overlap. This configuration suggests that wild germplasm in the collection includes both clearly differentiated backgrounds and internally heterogeneous subsets, which is relevant for pre-breeding design and parent selection.

The gel-based observations and matrix-based results were coherent: representative ScoT and SRAP profiles showed both shared and differential bands, and this signal translated into stable group structure after binary scoring and multivariate analysis. In practical terms, this supports the use of carefully filtered dominant marker data as a reliable strategy for germplasm stratification in Hordeum collections.

A methodological limitation is intrinsic to dominant markers: they do not separate homozygous presence from heterozygous presence at a locus. Therefore, while group-level patterns appear robust, finer-scale inference would benefit from complementary codominant or sequence-based datasets in future work.

The clear genetic differentiation observed between cultivated barley (*H. vulgare*) and wild *H. bulbosum* accessions in the present study has practical implications for breeding strategies that rely on the Bulbosum method for haploid and doubled haploid (DH) production. The Bulbosum system is based on interspecific hybridization between *H. vulgare* and *H. bulbosum*, followed by selective chromosome elimination and it has been developed and applied in Romanian barley breeding programs at NARDI Fundulea.

Pioneering studies by Alexandrina Mihăilescu and Aurel Giura demonstrated the feasibility of haploid production via *H. bulbosum* crosses, evaluated cross-compatibility between Romanian barley cultivars and *H. bulbosum*, and optimized embryo rescue and plant regeneration protocols (Giura and

Mihăilescu, 1998). Their work established the *Bulbosum* method as a practical tool for rapid homozygosity and accelerated line development in barley breeding. More recent contributions by Leonard-Alexandru Dumitru (Dumitru, 2024) have documented continued implementation of doubled haploid technologies within the Fundulea breeding pipeline, confirming the long-term integration of *H. bulbosum* mediated DH production into applied improvement programs.

Taken together, the structured diversity detected in this study reinforces the strategic importance of maintaining and molecularly characterizing *H. bulbosum* germplasm. When combined with established DH technologies developed at Fundulea, ScoT and SRAP profiling contributes to a more targeted and efficient utilization of wild barley resources in modern breeding programs.

CONCLUSIONS

SCoT and SRAP markers, provided robust and biologically meaningful resolution of diversity among the 50 Hordeum genotypes. The combined analysis clearly separated cultivated genotypes from wild *H. bulbosum* accessions, identified a distinct Hb4X subgroup and revealed substantial structure within the diploid wild pool. These results support the utility of wild *Hordeum* resources in pre-breeding and confirm that combined dominant-marker profiling is a practical tool for parent selection and germplasm organization.

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REFERENCES

Abaza, N.O., Yousief, S.S., Moghaieb, R.E., 2022. *The efficiency of SCoT, ISSR, and SRAP markers for detecting genetic polymorphism among Egyptian barley genotypes*. Journal of Pharmac. Negative Results: 1851-1863.

Abouseada, H.H., Mohamed, A.S.H., Teleb, S.S., Badr, A., Tantawy, M.E., Ibrahim, S.D., Ellmouni, F.Y., Ibrahim, M., 2023. *Genetic diversity analysis in wheat cultivars using SCoT and ISSR markers, chloroplast DNA barcoding and grain SEM*. BMC Plant Biology, 23(1): 193.

Almarri, N.B., Alghamdi, S.S., ElShal, M.H., Afzal, M., 2023. *Estimating genetic diversity among durum wheat (Triticum durum desf.) landraces using morphological and SRAP markers*. Journal of the Saudi Society of Agricultural Sciences, 22(5): 273-282.

Almerekova, S., Genievskaya, Y., Abugalieva, S., Sato, K., Turuspekov, Y., 2021. *Population structure and genetic diversity of two-rowed barley accessions from Kazakhstan based on SNP genotyping data*. Plants, 10(10), 2025.

Badr, A., Sch, R., Rabey, H.E., Effgen, S., Ibrahim, H.H., Pozzi, C., Rohde, W., Salamini, F., 2000. *On the origin and domestication history of barley (Hordeum vulgare)*. Molecular Biology and Evolution, 17(4): 499-510.

Bothmer, R., Jacobsen, N., Baden, C., Jorgensen, R.B., Linde-Laursen, I., 1995. *An ecogeographical study of the genus Hordeum (2nd edition)*. 129 p, ISBN: 978-92-9043-229-6, ISBN: 92-9043-229-2.

Chapman, E.A., Thomsen, H.C., Tulloch, S., Correia, P.M.P., Luo, G., Najafi, J., DeHaan, L.R., Crews, T.E., Olsson, L., Lundquist, P.-O., Westerbergh, A., Pedas, P.R., Knudsen, S., Palmgren, M., 2022. *Perennials as Future Grain Crops: Opportunities and Challenges*. Frontiers in Plant Science, 13, 898769.

Collard, B.C., and Mackill, D.J., 2009. *Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants*. Plant Molecular Biology Reporter, 27: 86-93.

Dabral, H., Singh, R.K., Baskheti, D.C., Singh, R., Goswami, A., Singh, A., 2022. *Assessment of molecular diversity among maize inbreds using Sequence Related Amplified Polymorphism (SRAP) Markers*. J. Plant Sci. Agri. Res., 6(3): 78.

Dido, A.A., Krishna, M.S.R., Assefa, E., Degefu, D.T., Singh, B.J.K., Tesfaye, K., 2022. *Genetic diversity, population structure and relationship of Ethiopian barley (Hordeum vulgare L.) landraces as revealed by SSR markers*. Journal of Genetics, 101(1), 9.

Dumitru, L.A., 2024. *Methodological approaches for producing doubled haploids in wheat and barley at NARDI Fundulea*. An. INCDA Fundulea, XCII: 45-58. https://www.incda-fundulea.ro/anale/92/92.8.pdf

Elakhdar, A., Kumamaru, T., Qualset, C.O., Brueggeman, R.S., Amer, K., Capo-chichi, L., 2018. *Assessment of genetic diversity in Egyptian barley (Hordeum vulgare L.) genotypes using SSR and SNP markers*. Genetic Resources and Crop Evolution, 65: 1937-1951.

El Haddad, N., Kabbaj, H., Zaïm, M., El Hassouni, K., Tidiane Sall, A., Azouz, M., Ortiz, R., Baum, M., Amri, A., Gamba, F., Bassi, F.M., 2021. *Crop wild relatives in durum wheat breeding: Drift or thrift?* Crop Science, 61(1): 37-54.

Elshafei, A.A., Ibrahim, E.I., Abdellatif, K.F., Salem, A.E.A.K., Moustafa, K.A., Al-Doss, A.A., Migdadi, H.M., Hussien, A.M., Soufan, W., Abd El Rahman, T., Eldemery, S.M., 2024. *Molecular and agro-morphological characterization of new barley genotypes in arid environments*. BMC Biotechnology, 24.

Fetch Jr, T., Johnston, P.A., Pickering, R., 2009. *Chromosomal location and inheritance of stem rust resistance transferred from Hordeum bulbosum into cultivated barley (H. vulgare)*. Phytopathology, 99(4): 339-343.

Galal, A.A., Safhi, F.A., El-Hity, M.A., Kamara, M.M., Gamal El-Din, E.M., Rehan, M., Farid, M., Behiry, S.I., El-Soda, M., Mansour, E., 2023. *Molecular genetic diversity of local and exotic durum wheat genotypes and their combining ability for agronomic traits under water deficit and well-watered conditions*. Life, 13(12), 2293.

Ghonaim, M.M., Attya, A.M., Aly, H.G., Mohamed, H.I., Omran, A.A., 2023. *Agro-morphological, biochemical, and molecular markers of barley genotypes grown under salinity stress conditions*. BMC Plant Biology, 23(1), 526.

Giura, A., and Mihăilescu, A., 1998. *Evaluation of pollinators (Zea mays L. and Hordeum bulbosum L.) for wheat and barley haploid production*. Rom. Agr. Res., 9-10: 41-48.

Güngör, H., Ilhan, E., Kasapoğlu, A.G., Filiz, E., Pour, A.H., Valchev, D., Valcheva, D., Haliloğlu, K., Dumlupinar, Z., 2022. *Genetic diversity and population structure of Barley cultivars released in Turkey and Bulgaria using iPBS-retrotransposon and SCoT markers*. Journal of Agricultural Sciences: 14-14.

Habiba, R., Bashasha, J., Haffez, S.H., Abo Leilah, A.A.A., 2021. *Assessment of genetic diversity using SCoT markers and some morphological traits in ten lines of barley (Hordeum vulgare L.)*. Assiut Journal of Agricultural Sciences, 52(4): 53-65.

Hayes, R.C., Wang, S., Newell, M.T., Turner, K., Larsen, J., Gazza, L., Anderson, J.A., Bell, L.W., Cattani, D.J., Frels, K., 2018. *The performance of early-generation perennial winter cereals at 21 sites across four continents*. Sustainability, 10(4), 1124.

Hoseinzadeh, P., Ruge-Wehling, B., Schweizer, P., Stein, N., Pidon, H., 2020. *High resolution mapping of a Hordeum bulbosum-derived powdery mildew resistance locus in barley using distinct homologous introgression lines*. Frontiers in Plant Science, 11, 225.

Hromadová, Z., Gálová, Z., Mikolášová, L., Balážová, Ž., Vivodík, M., Chňapek, M., 2023. *Efficiency of RAPD and SCoT markers in the genetic diversity assessment of the common bean*. Plants, 12(15), 2763.

Johnston, P.A., Niks, R.E., Meiyalaghan, V., Blanchet, E., Pickering, R., 2013. *Rph22: mapping of a novel leaf rust resistance gene introgressed from the non-host Hordeum bulbosum L. into cultivated barley (Hordeum vulgare L.)*. Theoretical and Applied Genetics, 126: 1613-1625.

Khaled, A.G.A., Elameen, T.M., Ahmed, A.Y.M., Mohiy, M., Elshazly, I.F.O., 2021. *SRAP molecular markers linked to three morphological traits in Egyptian bread wheat (Triticum aestivum L.)*. SVU-International Journal of Agricultural Sciences, 3(3): 145-158.

Khoury, C.K., Brush, S., Costich, D.E., Curry, H.A., De Haan, S., Engels, J.M., Guarino, L., Hoban, S., Mercer, K.L., Miller, A.J., Nabhan, G.P., 2022. *Crop genetic erosion: understanding and responding to loss of crop diversity*. New Phytologist, 233(1): 84-118.

Li, G., and Quiros, C.F., 2001. *Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica*. Theoretical and Applied Genetics, 103: 455-461.

Ma, X., Tian, H., Minaeifar, A.A., 2022. *SCoT Molecular Markers and Genetic Diversity of Stellaria media (L.) Vill*. Cytologia, 87(1): 23-28.

Mohammed, N.A., Afzal, M., Al-Faifi, S.A., Khan, M.A., Refay, Y.A., Al-Samin, B.H., Alghamdi, S.S., Ibrahim, A., 2023. *Effectiveness of sequence-related amplified polymorphism (SRAP) markers to assess the geographical origin and genetic diversity of collected lentil genotypes*. Plant Biotechnology Reports, 17(4): 519-530.

Pickering, R.A., Hill, A.M., Michel, M., Timmerman-Vaughan, G.M., 1995. *The transfer of a powdery mildew resistance gene from Hordeum bulbosum L. to barley (H. vulgare L.) chromosome 2 (2I)*. Theoretical and Applied Genetics, 91: 1288-1292.

Pidon, H., Ruge-Wehling, B., Will, T., Habekuß, A., Wendler, N., Oldach, K., Maasberg-Prelle, A., Korzun, V., Stein, N., 2024. *High-resolution mapping of Ryd4Hb, a major resistance gene to Barley yellow dwarf virus from Hordeum bulbosum*. Theoretical and Applied Genetics, 137(3): 1-16.

Ruge, B., Linz, A., Pickering, R., Proeseler, G., Greif, P., Wehling, P., 2003. *Mapping of Rym14 Hb, a gene introgressed from Hordeum bulbosum conferring resistance to BaMMV and BaYMV in barley*. Theoretical and Applied Genetics, 107: 965-971.

Saisho, D., and Purugganan, M.D., 2007. *Molecular phylogeography of domesticated barley traces expansion of agriculture in the Old World*. Genetics, 177(3): 1765-1776.

Scholz, M., Ruge-Wehling, B., Habekuß, A., Schrader, O., Pendinen, G., Fischer, K., Wehling, P., 2009. *Ryd4 Hb: a novel resistance gene introgressed from Hordeum bulbosum into barley and conferring complete and dominant resistance to the barley yellow dwarf virus*. Theoretical and Applied Genetics, 119: 837-849.

Serpoush, M., Garcia-Cozar, F., Salayeva, S., Ojaghi, J., 2022. *Molecular markers efficiency for assessment of genetic structure in barley accessions*. Cytology and Genetics, 56(6): 548-558.

Shaban, A.S., Arab, S.A., Basuoni, M.M., Abozahra, M.S., Abdelkawy, A.M., Mohamed, M.M., 2022. *SCoT, ISSR, and SDS-PAGE Investigation of genetic diversity in several egyptian wheat genotypes under normal and drought conditions*. International Journal of Agronomy, 2022(1), 7024028.

Shtaya, M.J.Y., Sillero, J.C., Flath, K., Pickering, R., Rubiales, D., 2007. *The resistance to leaf rust and powdery mildew of recombinant lines of barley (Hordeum vulgare L.) derived from H. vulgare× H. bulbosum crosses*. Plant Breeding, 126(3): 259-267.

Singh, A.K., Rossnagel, B.G., Scoles, G.J., Pickering, R.A., 2004. *Identification of a quantitatively inherited source of Hordeum bulbosum derived scald resistance from barley line 926K2/11/1/5/1*. Canadian Journal of Plant Science, 84(3): 935-938.

Tahir, N., Lateef, D.D., Rasul, K.S., Rahim, D., Mustafa, K.M., Sleman, S., Mirza, A., Aziz, R., 2023. *Assessment of genetic variation and population structure in Iraqi barley accessions using ISSR, CDDP and SCoT markers*. Czech Journal of Genetics and Plant Breeding, 59(2023), p.3.

Walther, U., Rapke, H., Proeseler, G., Szigat, G., 2000. *H. bulbosum-a new source of disease resistance-transfer of resistance to leaf rust and mosaic viruses from H. bulbosum into winter barley*. Plant Breeding, 119(3): 215-218.

Wendler, N., Mascher, M., Nöh, C., Himmelbach, A., Scholz, U., Ruge-Wehling, B., Stein, N., 2014. *Unlocking the secondary gene-pool of barley with next-generation sequencing*. Plant Biotechnology Journal, 12(8): 1122-1131.

Westerbergh, A., Lerceteau-Köhler, E., Sameri, M., Bedada, G., Lundquist, P.-O., 2018. *Towards the Development of Perennial Barley for Cold Temperate Climates-Evaluation of Wild Barley Relatives as Genetic Resources*. Sustainability, 10(6), 1969.

Yirgu, M., Kebede, M., Feyissa, T., Lakew, B., Woldeyohannes, A.B., Fikere, M., 2023. *Single nucleotide polymorphism (SNP) markers for genetic diversity and population structure study in Ethiopian barley (Hordeum vulgare L.) germplasm*. BMC Genomic Data, 24(1), 7.

Yu, X., Casonato, S., Jones, E., Butler, R.C., Johnston, P.A., Chng, S., 2022. *Phenotypic characterization of the Hordeum bulbosum derived leaf rust resistance genes Rph22 and Rph26 in barley*. Journal of Applied Microbiology, 133(3): 2083-2094.

Zagorcheva, T., Rusanov, K., Bosmali, E., Savvides, A., Madesis, P., Fotopoulos, V., Rusanova, M., Ustabashiev, F., Atanassov, I., 2024. *SRAP markers for characterization of the genetic diversity and differentiation of Pinus nigra populations in protected forested areas in Bulgaria, Greece and Cyprus*. Biotechnology & Biotechnological Equipment, 38(1), 2331192.

Zhang, F., and Batley, J., 2020. *Exploring the application of wild species for crop improvement in a changing climate*. Current Opinion in Plant Biology, 56: 218-222.

Zohary, D., Hopf, M., Weiss, E., 2012. *Domestication of plants in the Old World*. 4th ed. Oxford: Oxford University Press. https://doi.org/10.1093/acprof:osobl/9780199549061.001.0001

SUPPLEMENTARY MATERIAL

Table S1. List of analyzed genotypes

No.	Genotype	Species	Group	No.	Genotype	Species	Group	No.	Genotype	Species	Group
1	Hb 3-2	*H. bulbosum*	Hb3	18	Hb 3 13-1	*H. bulbosum*	Hb3	35	Hb 4-127	*H. bulbosum*	Hb4
2	Hb 3-3	*H. bulbosum*	Hb3	19	Hb 3 13-2	*H. bulbosum*	Hb3	36	Hb 4-128	*H. bulbosum*	Hb4
3	Hb 3-4	*H. bulbosum*	Hb3	20	Hb 4-31	*H. bulbosum*	Hb4	37	Hb 4-129	*H. bulbosum*	Hb4
4	Hb 3-5	*H. bulbosum*	Hb3	21	Hb 4-73	*H. bulbosum*	Hb4	38	Hb 4-131	*H. bulbosum*	Hb4
5	Hb 3-6	*H. bulbosum*	Hb3	22	Hb 4-74	*H. bulbosum*	Hb4	39	Hb 4-136	*H. bulbosum*	Hb4
6	Hb 3-9	*H. bulbosum*	Hb3	23	Hb 4-79	*H. bulbosum*	Hb4	40	Hb 4x-132	*H. bulbosum*	Hb4X
7	Hb 3-12	*H. bulbosum*	Hb3	24	Hb 4-82	*H. bulbosum*	Hb4	41	Hb 4x-133	*H. bulbosum*	Hb4X
8	Hb 3-14	*H. bulbosum*	Hb3	25	Hb 4-85	*H. bulbosum*	Hb4	42	Hb 4x-134	*H. bulbosum*	Hb4X
9	Hb 3-15	*H. bulbosum*	Hb3	26	Hb 4-90	*H. bulbosum*	Hb4	43	Hb 4x-135	*H. bulbosum*	Hb4X
10	Hb 3-19	*H. bulbosum*	Hb3	27	Hb 4-92	*H. bulbosum*	Hb4	44	Hb 4x-137	*H. bulbosum*	Hb4X
11	Hb 3-20	*H. bulbosum*	Hb3	28	Hb 4-112	*H. bulbosum*	Hb4	45	CC 21-03	*H. vulgare*	cultivated
12	Hb 3-25	*H. bulbosum*	Hb3	29	Hb 4-114	*H. bulbosum*	Hb4	46	CC 21-05	*H. vulgare*	cultivated
13	Hb 3-35	*H. bulbosum*	Hb3	30	Hb 4-116	*H. bulbosum*	Hb4	47	CC 01-04	*H. vulgare*	cultivated
14	Hb 3-63	*H. bulbosum*	Hb3	31	Hb 4-118	*H. bulbosum*	Hb4	48	CC 01-05	*H. vulgare*	cultivated
15	Hb 3-66	*H. bulbosum*	Hb3	32	Hb 4-123	*H. bulbosum*	Hb4	49	CC 01-08	*H. vulgare*	cultivated
16	Hb 3-67	*H. bulbosum*	Hb3	33	Hb 4-124	*H. bulbosum*	Hb4	50	CC 01-09	*H. vulgare*	cultivated
17	Hb 3-68	*H. bulbosum*	Hb3	34	Hb 4-126	*H. bulbosum*	Hb4				

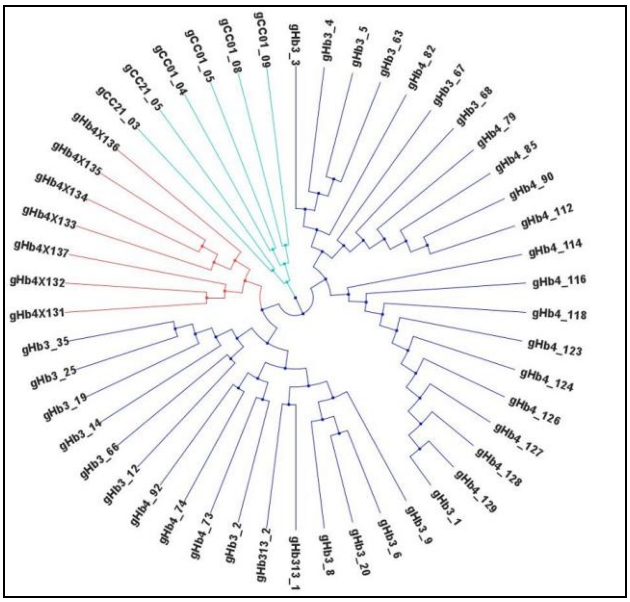

Figure S1. UPGMA dendrogram based on SCoT markers. The tree was constructed from the SCoT presence/absence matrix to evaluate genetic structure captured by SCoT alone.

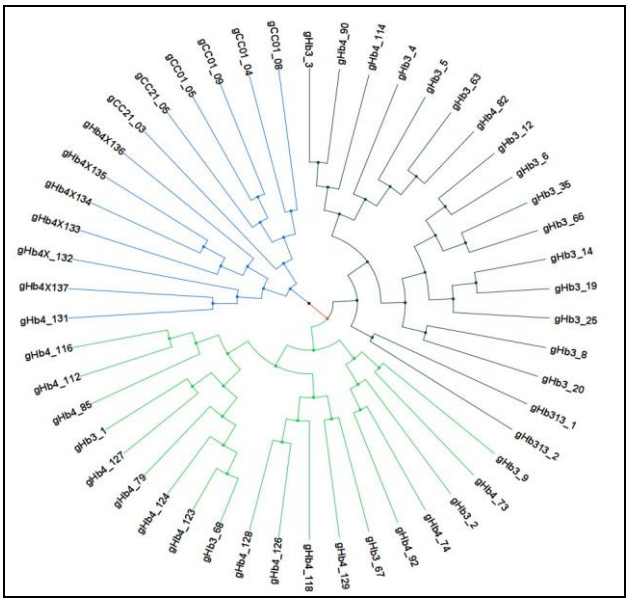

Figure S2. UPGMA dendrogram based on SRAP markers. The tree was constructed from the SRAP presence/absence matrix to evaluate genetic structure captured by SRAP alone.

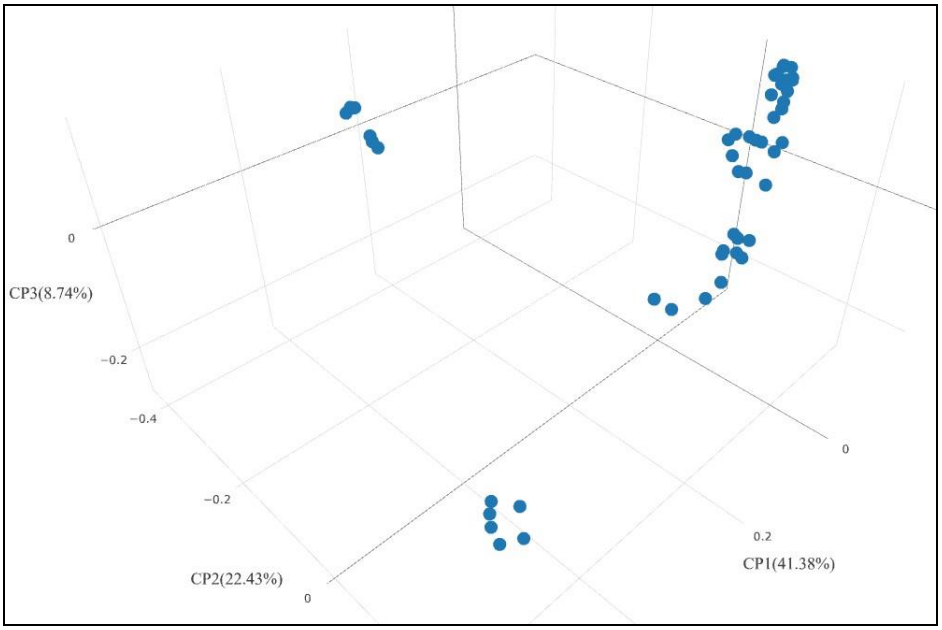

Figure S3. Three-dimensional PCA based on the combined SCoT and SRAP binary matrix