Inter Simple Sequence Repeat Data Reveals The Physical Characteristics of *Helleborus*

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Abstract

Helleborus is an evergreen plant belongs to *Ranunculaceae*, it is commonly known as "Christmas Rose", "Winter Rose" and "Lenten Rose". ISSR markers are highly polymorphic and are useful for studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology. We conducted a characteristic investigation and inter simple sequence repeat analysis for use as breeding parents through the classification of 17 *Helleborus* germplasm. In the present study, we used 100 ISSR primers for analysis of genetic diversity in *Helleborus*, and 16 primers produced highly polymorphic DNA fragments. As per results, sixteen primers ((AG)$_8$T, (GA)$_8$T, (GA)$_8$C, (CT)$_8$A, (CT)$_8$G, (GA)$_8$YT, (GA)$_8$YC, (CT)$_8$RC, (CA)$_8$RT, (GT)$_8$YC, (TC)$_8$RA, (TC)$_8$RT, (AC)$_8$YG, (TG)$_8$RT, (CTTCA)$_3$ and (GGAGA)$_3$) generated 100% polymorphic patterns in tested 17 *Helleborus* germplasm. Nei's genetic diversity and Shannon's information index had their highest value in primer (CA)$_8$RT as 0.400 and 0.581, respectively, and the lowest value in primer (CTTCA)$_3$ as 0.199 and 0.377, respectively. Based on these acquired genotypes of 16 ISSR primers, we analyzed the genetic relationship of 17 *Helleborus* germplasm, and the tested germplasm was classified into three groups at genetic distance value of 0.22. According these results we suggested that valid guidelines for the collection and conservation of *Helleborus* genetic resources.

Results

Table 1. Genetic variability across all the accessions of *Helleborus* using ISSR marker

Locus	ISSR	Np*	H**	I***
807	(AG)$_8$T	10	0.284	0.446
810	(GA)$_8$T	9	0.389	0.572
811	(GA)$_8$C	10	0.306	0.468
814	(CT)$_8$A	5	0.216	0.357
815	(CT)$_8$G	11	0.351	0.523
840	(GA)$_8$YT	13	0.256	0.407
841	(GA)$_8$YC	8	0.313	0.476
844	(CT)$_8$RC	10	0.321	0.493
846	(CA)$_8$RT	8	0.4	0.581
850	(GT)$_8$YC	9	0.311	0.468
852	(TC)$_8$RA	7	0.221	0.357
853	(TC)$_8$RT	10	0.35	0.521
857	(AC)$_8$YG	9	0.329	0.497
858	(TG)$_8$RT	7	0.308	0.467
879	(CTTCA)$_3$	5	0.199	0.337
880	(GGAGA)$_3$	8	0.33	0.499
Total		139	0.311	0.474

* Np = Number of scored and polymorphic bands
**H = Nei's (1973) gene diversity
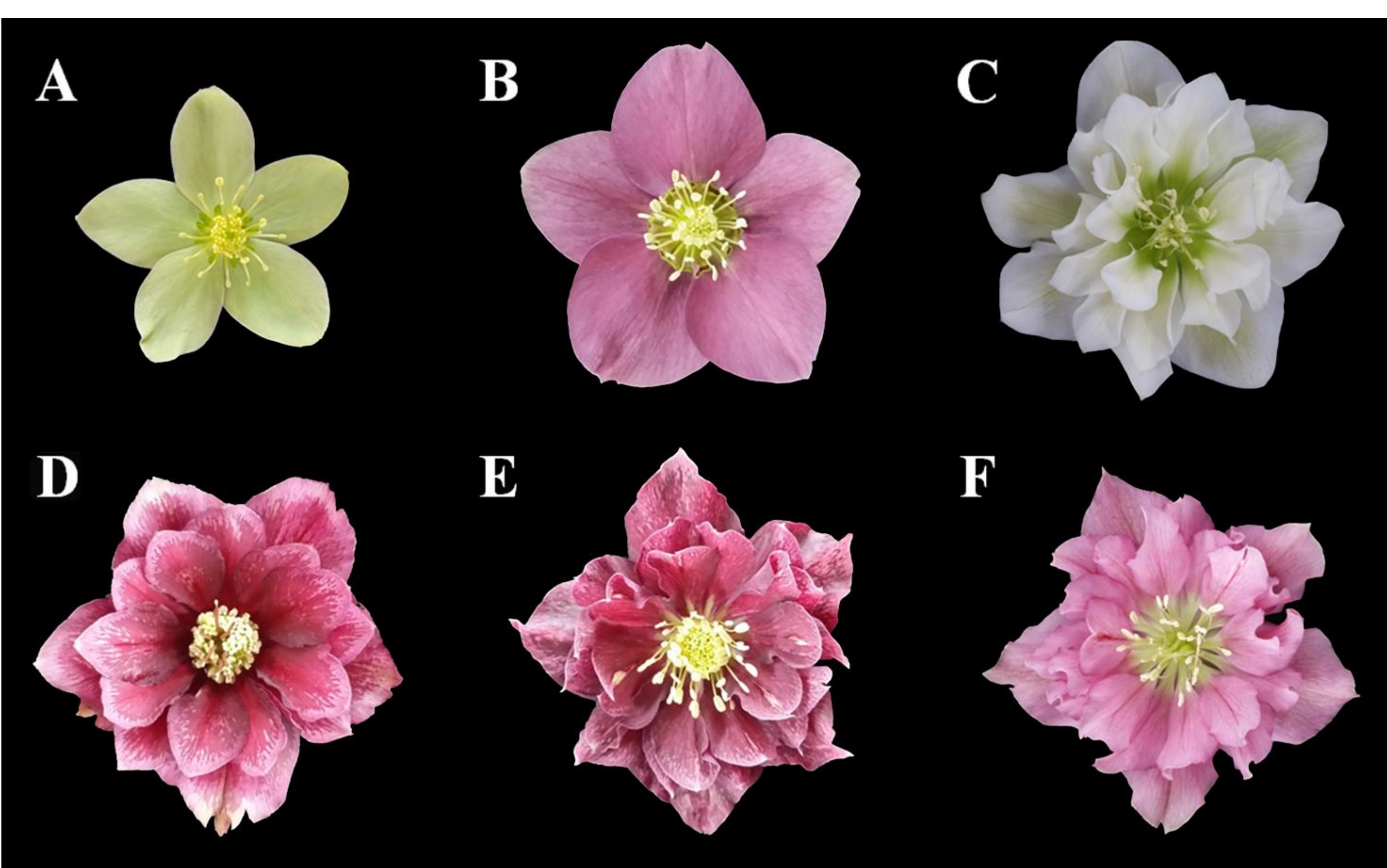*** I = Shannon's Information index [Lewontin (1972)]

Fig. 1. Relationship comparison of morphological characteristics and ISSR analysis. A: *Helleborus argutifolius*, B: "red hybride" C: "double white", D: "double ellen red", E: "double red", F: "double purple".
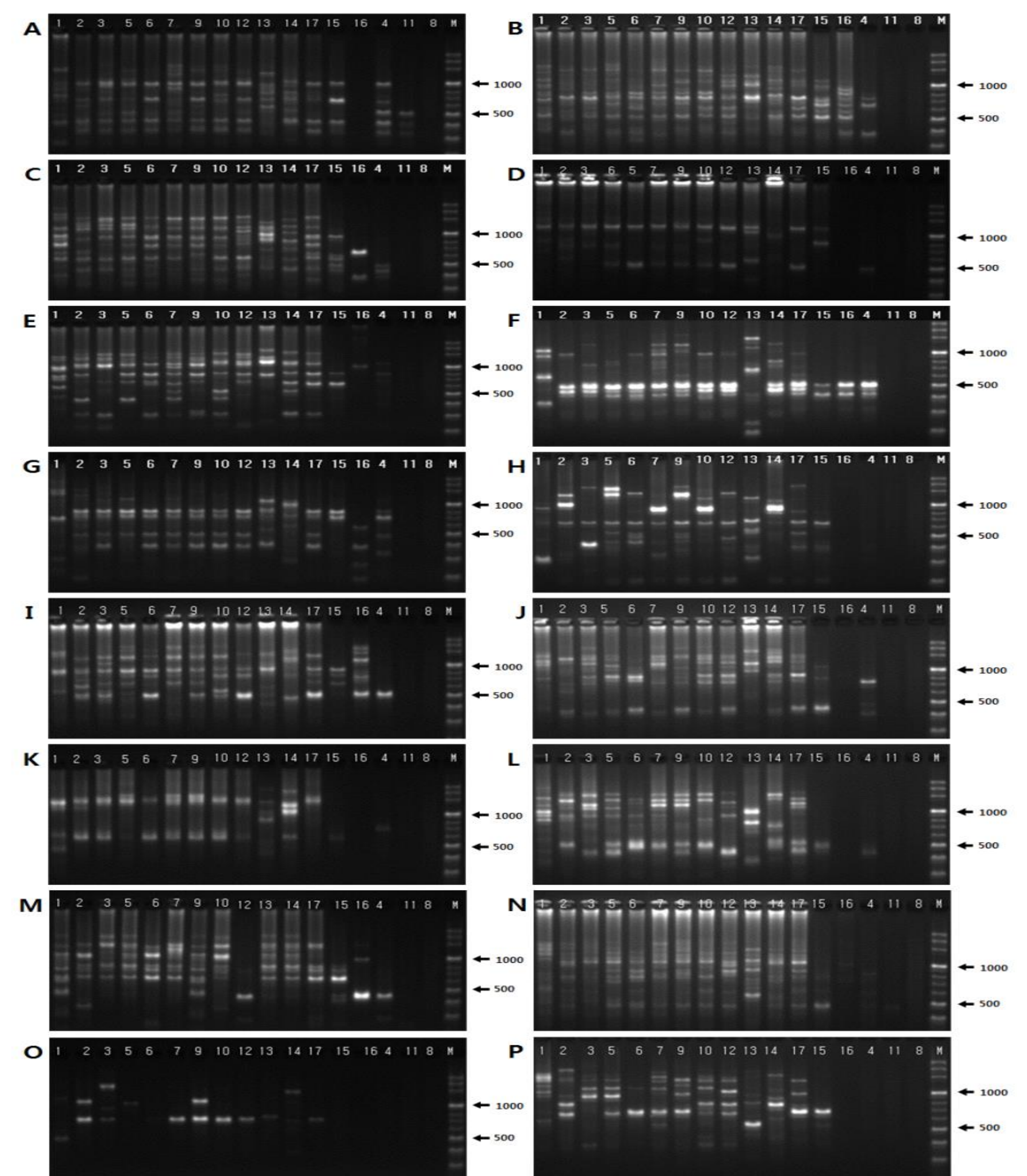

Fig. 2. ISSR-PCR band profiles generated by used 16 primer in 17 *Helleborus* species.
ISSR 16 primer; A: 807, B: 810, C: 811, D: 814, E: 815, F: 840, G: 841, H: 844, I: 846, J: 850, K: 852, L: 853, M: 857, N: 858, O: 879, P: 880. Lane 1: *Helleborus niger*, lane 2: "yellow lady", lane 3: "white", lane 4: "orient", lane 5: "double picotee", lane 6: "pink spotted", lane 7: "double purple", lane 8: "double red", lane 9: "double white", lane 10: "pink lady", lane 11: "red hybride", lane 12: "white lady", lane 13: *Helleborus argutifolius*, lane 14: "double ellen purple", lane 15: "double ellen pink", lane 16: "double ellen white", lane 17: "double ellen red" and M: Weight maker.
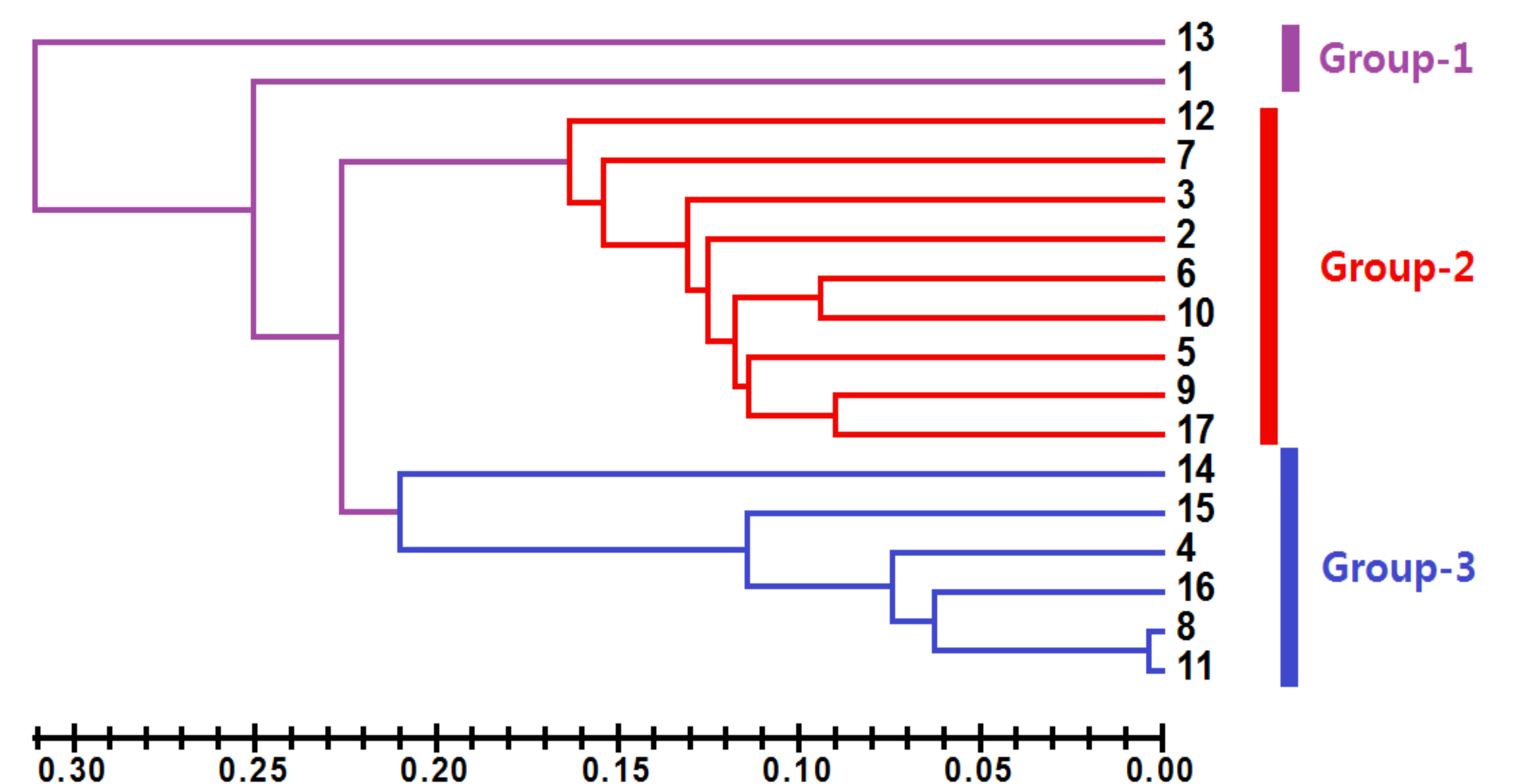

Fig. 3. Dendrogram generated by using UPGMA cluster analysis based on genetic diversity of *Helleborus* accessions.

Conclusions

In this study, ISSR marker result showed that of comprison analysis on dendrogram indicating the variations among the genetic distance and morphological properties of *Helleborus*. Compared the results between the "Double white" and "Double ellen red" varieties, genetic distance is proximity to 0.09 and showed differences in morphological characteristics on flower color is white and purple. In additions, result of comparing the "Double red" and "Double purple" varieties, genetic distance is far to 0.23, but showed simmilar in morphorlogical characteristics on flower color is brightly purple. This results suggest that, using method ISSR marker is considered will help to study more appropriate species classification than shape classification.

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