Horticulture, Environment, and Biotechnology (2024) 65:1043–1052
https://doi.org/10.1007/s13580-024-00629-z

RESEARCH REPORT

Evaluation of genetic diversity using simple sequence repeat markers and analysis of cross compatibility in hydrangeas

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Received: 20 September 2023 / Revised: 17 June 2024 / Accepted: 17 June 2024 / Published online: 5 August 2024

Abstract

Hydrangeas with large inflorescences are widely used as cut flowers and in floral arrangements. However, the genetic diversity of commercially grown hydrangeas has been limited owing to the breeding focus on popular species. Therefore, this study was conducted to suggest interspecific breeding strategies for expanding the genetic diversity in *Hydrangea L.* by evaluating genetic diversity in the seven main species collected in Korea and analyzing cross compatibility via intra- and interspecific hybridization. Interspecific diversity evaluation using simple sequence repeat markers resulted in the segregation of 35 varieties accounting for seven species into five groups as follows: (1) *Hydrangea paniculate* group, (2) *Hydrangea arborescnes* group, (3) *Hydrangea anomala* and *Hydrangea aspera* group, (4) *Hydrangea quercifolia* group, and (5) *Hydrangea macrophylla* and *Hydrangea serrata* group. Cross compatibility was confirmed via intra- and interspecific crossing, and hybrids were obtained in 18 crossing combinations. Intraspecific hybrids tend to be easy to obtain, but interspecific hybrids are difficult to obtain due to a variety of factors. While most of the interspecific hybrids were obtained using ovule culture, the crossing between *H. macrophylla* and *H. serrata* created hybrids from seed sowing, indicating that *H. serrata* is a subspecies of *H. macrophylla*. Bilateral and unilateral incompatibilities were observed across the obtained hybrids. Particularly, weak bilateral compatibility was observed between *H. serrata* and *H.paniculata*. Accordingly, it was proposed that hybrids between *H. macrophylla* and *H. serrata* can be successfully used as parental materials in crossing with *H. paniculata* to improve cold tolerance. In addition, cross compatibility was improved in interspecific crossing using *H. macrophylla* and *H. arborescens* as maternal plants. It is anticipated that these finding will help improve the genetic diversity in commercial hydrangeas.

Keywords Cross breeding · Germplasm · Cold hardiness · Hybridization · Unilateral incompatibility

1 Introduction

Hydrangeas with large inflorescences are popular as for cut flowers and used in floral arrangements. Hydrangeas account for approximately one hundred billion dollars in annual shrub sales in the United States; it is a major flower crop that ranks first in potted plant sales and is in the top 10 in cut flower sales in Korea (Owen et al. 2016; aT 2021). The sepals of hydrangeas come in white and pink colors. White sepals usually remain unchanged. Pink sepals changes to purple and blue depending on the aluminum uptake rate from the soil.

Hydrangeas, which belong to the *Hydrangeaceae* family and *Hydrangea* genus, can be classified into 23–70 species based on the taxonomic method used (McClintock 1957; Sandra et al. 2015). The species mainly distributed in Korea include *Hydrangea macrophylla*, *Hydrangea. serrata*, *Hydrangea arborescens*, *Hydrangea paniculate*, *Hydrangea quercifolia*, *Hydrangea anomala*, and *Hydrangea aspera*. The National Seed Resources classifies hydrangeas into three types (round, flat, and conical) according to the shape of their inflorescence (KSVS 2015). Hydrangea species show diversity in leaf shape, presence of hair on the leaf surface, number of sepals (single flower or double flower), sepal color, resistance to low and high temperatures, and various agricultural characteristics (resistance to pests and

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diseases). Although the seven aforementioned species have different morphological and agricultural characteristics, previous efforts have focused on the breeding of the more popular *H. macrophylla* and *H. serrata* species. In addition, the breeding of both species focuses on the enlargement of flower size and diversification of sepal colors among various characteristics (Lawson-Hall and Rothera 2005).

Approximately 1000 cultivars have been developed, most of which belong to *H.macrophylla* and *H. serrata* species (Owen et al. 2016). Although many cultivars have been developed, most cultivars have been created via intraspecific crossing with similar breeding aims (Van Gelderen and Van Gelderen 2004). Recently, the popularity of *H. arborescens*, *H. paniculata*, and *H. quercifolia* has increased with the increase in interest in gardening; accordingly, the development of additional varieties has been attempted. *H. quercifolia* has been selected via open pollination (Reed 2004). Except for the two popular species (*H. macrophylla* and *H. serrata*), cultivars of other species are relatively scarce, as is the narrow morphological diversity (flower shape, flower color, plant type, etc.). Therefore, it is necessary to introduce novel traits via various crossings to overcome these problems and develop additional robust carefree hydrangea cultivars.

Cross breeding represents the main method of cultivar development via new genetic combinations between plants containing different traits, and interspecific hybridization is used to overcome the limitations of intraspecific hybridization. However, seedling death was reported in many cases or the crossing failed owing to other various factors. Typical factors that affect hybridization failure include different chromosome numbers, genome size, and pre- or post-fertilization barriers (Van Tuyl et al. 1990; Kim et al. 2001; Rinehart et al. 2006). Nevertheless, several studies have reported interspecific hybridization in hydrangeas, and seedlings have been obtained via seed sowing or ovule culture (Jones and Reed 2006; Kudo et al. 2008; Cai et al. 2015; Bak and Han 2022, 2023). Reed (2000) conducted a study on the cross-compatibility in hydrangeas to identify such factors and overcome these challenges to maximize breeding efficiency. Although the intra- and interspecific hybridization efficiencies were determined, the number of cultivars and species used was limited. Mendoza et al. (2013) analyzed cross-compatibility according to the genetic distance between species. This study selected suitable species for interspecific cross-breeding and proposed an interspecific hybridization strategy. Although several studies have been conducted, the cytological and genetic information required to improve the breeding efficiency of hydrangeas remains limited.

Randomly amplified polymorphic DNA and simple sequence repeat (SSR) markers have been used for analyzing genetic diversity to establish crossing strategies (Rinehart et al. 2006; Reed and Rinehart 2009; Khaing et al. 2018). SSR markers are useful for analyzing the genetic diversity of plant species owing to high economic efficiency, random occurrence throughout the entire genome, and high reproducibility. Consequently, it has been used to analyze the genetic relationship among 114 varieties of *Hydrangea* L. as well as 37 varieties of *H. paniculata* (Reed and Rinehart 2007, 2009). SSR markers have also been used to evaluate the genetic diversity among 14 species of *Hydrangea* L. and consequently, this breeding strategy was suggested for assessing vital traits or reconfirming previous classifications (Rinehart et al. 2006). However, many evaluations of genetic diversity using SSR markers have been limited to analyses that suggest breeding strategies or focus on *H. macrophylla* and *H. serrata*. Furthermore, SSR markers are more often utilized for the purpose of confirming hybridization and for identifying mislabeled cultivars.

The present study was performed to overcome the limitations in cultivar development owing to limited germplasm and to develop various hydrangea cultivars by increasing genetic diversity. We revealed the genetic diversity using SSR markers and an analysis of cross- compatibility via intra- and interspecific hybridization, which targeted seven species, to suggest appropriate breeding strategies.

2 Materials and methods

In total, 66 varieties accounting for seven species cultivated at the green houses at Chonnam National University were used in this study. Additionally, 35 varieties accounting for seven species were used for genetic diversity evaluation (Table 1). The plants were transplanted in plastic pots (30 cm) and grown in nutrient solutions according to the standard cultivation method (RDA 2022). The plants were cultivated under 70–80% shade during the crossing period.

Intraspecies and interspecific crossings were performed from 2018 to 2022. For pollination, the green and incomplete flowers which were not in full bloom were removed completely from the inflorescence, which consisted of complete and incomplete flowers. Subsequently, 50–100 complete flowers were left, and the others were removed to mature for pollination. The complete flowers of the mature inflorescence were emasculated using a pair of fine-tipped forceps before the flowers opened. Immediately, the inflorescence was covered with a paper bag to prevent entry of other pollen. Approximately 3 d after emasculation, pollination was performed when the stigma opened and its color changed to ivory. The dehiscing anthers were then touched directly to the exposed stigmas of the emasculated flowers. Pollinated inflorescences were covered with a paper bag, which was removed after 3 d. The pollen of *H. macrophylla* with the earliest flowering time was collected and used that year. Pollen of other species was collected in

Table 1 Characteristics of 35 Hydrangea taxa evaluated with single sequence repeat (SSR) markers

Accessions	Species[z]	Flower type[y]	Sepal type[x]	Leaf type[w]	Remontant[v]
JNHM01	*H. macrophylla*	Mophead	S	A	N
JNHM02	*H. macrophylla*	Mophead	S	A	N
JNHM03	*H. macrophylla*	Mophead	S	A	N
JNHM04	*H. macrophylla*	Mophead	S	A	N
JNHM05	*H. macrophylla*	Mophead	D	A	N
JNHM06	*H. macrophylla*	Mophead	S	A	N
JNHM07	*H. macrophylla*	Mophead	S	A	N
JNHM08	*H. macrophylla*	Mophead	S	A	N
JNHM09	*H. macrophylla*	Mophead	S	A	N
JNHS01	*H. serrata*	Lacecap	S	B	N
JNHS02	*H. serrata*	Lacecap	S	B	N
JNHS03	*H. serrata*	Lacecap	S	B	N
JNHQ03	*H. quercifolia*	Con-head	S	G	N
JNHQ02	*H. quercifolia*	Con-head	S	G	N
JNHQ01	*H. quercifolia*	Con-head	S	G	N
JNHQ04	*H. quercifolia*	Con-head	S	G	N
JNHA01	*H. arborescens*	Mophead	S	C	Y
JNHA02	*H. arborescens*	Mophead	S	C	Y
JNHA03	*H. arborescens*	Mophead	S	C	Y
JNHA04	*H. arborescens*	Mophead	S	C	Y
JNHA05	*H. arborescens*	Mophead	S	C	Y
JNHA06	*H. arborescens*	Mophead	S	C	Y
JNHP01	*H. paniculata*	Con-head	S	E	N
JNHP02	*H. paniculata*	Con-head	S	E	N
JNHP03	*H. paniculata*	Con-head	S	E	N
JNHP04	*H. paniculata*	Con-head	S	E	N
JNHP05	*H. paniculata*	Con-head	S	E	N
JNHP06	*H. paniculata*	Con-head	S	E	N
JNHP07	*H. paniculata*	Con-head	S	E	N
JNHP08	*H. paniculata*	Con-head	S	E	N
JNHP09	*H. paniculata*	Con-head	S	E	N
JNHP10	*H. paniculata*	Con-head	S	E	N
JNHPL01	*H. anomala*	Lacecap	S	F	N
JNHPL02	*H. anomala*	Lacecap	S	F	N
JNHAS01	*H. aspera*	Lacecap	S	D	N

[z]This is the species classified through investigation of crop characteristics based on estimated species information by the provider and botanical characteristics

[y]These were classified according to the Korea Seed & Variety Service Guidelines for Investigation of Crop Characteristics (*Hydrangeaspp*.)

[x]S denotes a single sepal with three to six sepals and "D" means a double sepal with more than six sepals. These were classified according to the Korea Seed & Variety Service Guidelines for Investigation of Crop Characteristics (*Hydrangeaspp*.)

[w]The leaf types according to the species are classified. Please refer to supplementary Fig. 1 for detailed information

[v]Remontant, which means reblooming potential was indicated by Y or N. Y means yes and N means no

1.5 mL tubes when freshly dehisced anthers were observed from the previous year. The lid of the 1.5 mL tube containing the collected pollen was sealed using a wrap; the tube was stored in a deep freezer at–74 °C and then defrosted at room temperature and used in the following year for pollination. Fertilized capsules were usually harvested at maturation in December when they turned brown. The harvested capsules were crushed, filtered using a sieve, placed in a paper bag, labeled, and stored in a refrigerator at 4 °C. The stored seeds were then sown.

In total, 100–150 harvested seeds were each sown on a piece of rock wool (10 × 10 × 2 cm; Cultilene, Tilburg, Netherlands), and then the germination rate was confirmed after 1 month in a light-exposed growth room at 25 °C. The germinated seedlings were transferred to 33 mm Jiffy pots (Jiffy 7; Jiffy^R, Zwijndrecht, Netherlands) for acclimation.

The capsules obtained from interspecific crossing were collected 8–9 weeks after pollination. The capsules were disinfected in 70% ethanol for 30 s, and then immersed in a 1.5% sodium hypochlorite solution containing one drop of Tween 20 for 15 min, followed by rinsing 2–3 times with sterile distilled water. The sterilized ovaries were cut lengthwise using a pair of fine-tipped forceps and a scalpel, and ovules containing white embryos were extracted and placed on plates containing half-strength Gamborg's B5 media. The media containing ovules were cultured under light for 24 h. The germinated seedlings were transferred to 33 mm Jiffy pots (Jiffy 7; Jiffy^R) for acclimation, and then acclimated seedlings were transferred to a greenhouse.

From 2018 to 2022, approximately 100,000 seeds obtained via inter- and intraspecific hybridization were sown and cultured; this resulted in the generation of 36,164 hybrids. The obtained hybrids were classified according to species unit parentage and used for cross-compatibility analysis based on species.

The genomic DNA (gDNA) of 35 varieties accounting for seven species was extracted from young leaves for the evaluation of genetic diversity in hydrangeas. gDNA was extracted using the Higene™ Genomic DNA Prep Kit (GD263-06; Solgent, Korea) according to the manufacturer's instructions. The extracted gDNA was subjected to electrophoresis on a 1.6% agarose gel. The gDNA concentration confirmed based on the intensity of the band under ultraviolet irradiation was recorded. Extracted gDNA was amplified using 68 SSR marker primers developed previously (supplementary Table 1). Polymerase chain reaction (PCR) assays were performed using the mixture prepared in a total volume of 20μL using the Solg™ e-Taq DNA Polymerase kit (SET15-R500; Solgent) by following the manufacturer's instructions. The PCR conditions were as follows: 3 min at 95 °C, 2 cycles of 1 min at 95 °C, 1 min at 60 °C, and then 27 cycles of 30 s at 35 °C, 30 s at 60 °C, 30 s at 68 °C, and finally 4 min at 68 °C. Subsequently, the mixture was stored at 4 °C. After amplification, 2 μL of each sample was loaded onto a 3% agarose gel and run in 1X TBE buffer at 75 V for 40 min. Gels were then stained and the reaction products were viewed. The DNA profiles were visually scored based on gel photographs. The presence of reaction products was scored as (1) and their absence was scored as (0). The data obtained from the SSR scoring were used to calculate a similarity matrix using the Jaccard coefficients (Jaccard 1901). Principal coordinate analysis (PCoA) and Unweighted Pair Group The method with Arithmetic mean (UPGMA) were performed to determine the genetic distances and relationships between varieties using Paleontological Statistics (PAST, ver. 3.15). Statistical support estimates for the resultant clusters were obtained from the UPGMA bootstrap analysis with 1,000 replications.

3 Results

3.1 Interspecific diversity evaluations

Diversity evaluations between 35 varieties consisting of seven species using SSR markers resulted in the species being divided into the following five groups: (1) *H.macrophylla* and *H.serrata*, (2) *H.quercifolia*, (3) *H.anomala* and *H.aspera*, (4) *H.arborescens*, and (5) *H.paniculata* (Fig. 1). Although the seven species used in the study were expected to be divided into seven groups, they were divided into five groups because *H.macrophylla* and *H.serrata* were divided into the same group, as were *H.anomala* and *H.aspera*. As a result of the PCoA, it was confirmed that *H.paniculata*, *H.arborescens*, and *H.macrophylla* and *H.serrata* were located at each angular point like a triangle, and each group had a significant genetic distance (Fig. 2). The *H.macrophylla* and *H.serrata* groups showed ranging intraspecies genetic diversity compared to other species as it was confirmed that the distribution density (genetic distance) between the varieties was higher than that of the other groups (Figs. 1, 2). The *H.quercifolia* group was genetically close to the *H.macrophylla* and *H.serrata* group and was located between the *H.anomala* and *H.aspera* group and the *H.macrophylla* and *H.serrata* group (Fig. 2). The *H.arborescens* group was genetically close to the *H.anomala* and *H.aspera* groups, and it was confirmed that the diversity was low because of the short genetic distance between the varieties within the species. *Hydrangeaanomala* and *H.aspera* were grouped together because they were contiguous to each other, even though they were not close to each other. The *H.paniculata* group showed a relatively distant separation from the other four groups. However, the genetic diversity in *H.paniculata* was similar because the genetic distance in the varieties was close.

3.2 Cross compatibility analysis

A total of 36,049 hybrids were created from 18 parentages and the number of hybrids was sorted by the parentage (Table 2). It was observed that crossing with all other species was possible when *H. macrophylla* was selected as the maternal plant. When *H. arborescens* was used as a maternal plant, it was possible to cross with five species. *Hydrangea macrophylla* and *H. arborescens* were identified as species which are relatively suited as maternal plants compared to

Table 2 Number of progenies according to cross combinations based on the hybridization results from 2018 to 2022

Type[z]	Parentage	No. pollinated flowers[y]	No. seedlings
Intra	*H. macrophylla* x *H.macrophylla*	≳9,000	32,730
Inter	*H. macrophylla* x *H. serrata*	≳300	780
Inter	*H. macrophylla* x *H. paniculata*	≳150	308
Inter	*H. macrophylla* x *H.arborescens*	≲500	2
Inter	*H. macrophylla* x *H. quercifolia*	≲700	24
Inter	*H. macrophylla* x *H. anomala*	≲250	8
Inter	*H. serrata* x *H. macrophylla*	≳100	448
Intra	*H. serrata* x *H. serrata*	≲30	2
Inter	*H. serrata* x *H. paniculate*	≲150	144
Inter	*H. arborescensxH. macrophylla*	≲300	3
Inter	*H. arborescens* x *H. serrata*	≲500	37
Inter	*H. arborescens* x *H. paniculata*	≳1,000	113
Intra	*H. arborescens* x *H.arborescens*	≲500	1,285
Inter	*H. arborescens* x *H. quercifolia*	≳500	26
Inter	*H. paniculata* x *H. serrata*	≲300	4
Intra	*H. paniculata* x *H. paniculata*	≳1,000	237
Inter	*H. paniculata* x *H. arborescens*	≲700	12
Intra	*H. quercifolia* x *H. quercifolia*	≲50	1
Total	18	≅16,030	36,164

[z]Intra means intraspecific hybridization and Inter means interspecific hybridization

[y]A flower that succeeds in crossing develops into an ovary. An ovary contains as little as one seed to as many as hundreds of seeds

other species. In the cases when *H. serrata* and *H. paniculata* were used as maternal plants, it was possible to obtain progenies from the three parentages respectably. When *H. quercifolia* was used as a maternal plant, it created only one progeny through intraspecies crossing. Progenies were not identified from parentage with different pollen parents.

Considering the frequency of crossing, it was confirmed that intraspecies crossing was generally good for seed formation from 2018 to 2022. Crossing focused on *H. macrophylla*, the most popular species, created the highest number of seedlings. It is noteworthy that by bilateral crossing *H.macrophylla* with *H.serrata*, numerous seedlings were obtained. In addition, the seedlings made through the bilateral crossing between *H.macrophylla* and *H.serrata* were gained through seed sowing, which is not an ovule culture. This indicates that *H.macrophylla* and *H.serrata* have high cross-compatibility. In the case of interspecific hybrids, 308 seedlings were secured using ovule culture when crossed between *H.macrophylla* as a maternal plant and *H.paniculata.* In contrast, there were no seedlings (Table 2). When *H.serrata* belonging to the same group as *H.macrophylla* was crossed with *H.paniculata*, 144 seedlings were obtained relatively easily. As a pollen donor, *H.serrata* was crossed with *H.paniculate*, and four seedlings were obtained. In addition, *H.serrata* and *H.paniculata* were bilaterally crossed. Based on various and numerous crossings, we have found that few cross combinations did not have complete incompatibility, and rather showed very low rate of compatibility by producing rare offsprings from unexpected cross combinations. It was confirmed that less than 10 plants were obtained from interspecific crosses.

Figure 3 shows whether more than 30 seedlings could be obtained resulting in the rare success and possibility of interspecific hybridization. Thirteen of the 49 cross combinations were successful. When *H. macrophylla* and *H. arborescens* are used in crossing as mother plants, interspecific hybridization with various species are achievable. In the case of crossing between *H. macrophylla* and *H. paniculata*, seedlings could not be obtained when *H. paniculata* was the maternal plant, thus complete unilateral incompatibility (UI) was confirmed. Likewise, UI was confirmed when *H. arborescens* was used as a pollen parent in the case of crossing between *H. arborescens* and *H. serrata*. Similarly, UI was confirmed in *H. anomala* x (crossed with) *H. macrophylla,* and *H. quercifolia* x *H. macrophylla*. *Hydrangea aspera* did not create seedlings in any combination. Except for the case of *H. macrophylla* x *H. anomala*, *H. anomala* was observed to be mostly incompatible as it was difficult to cross with other species.

4 Discussions

4.1 Interspecific Diversity Evaluations

Hydrangea macrophylla and *H.serrata* were grouped in the same cluster; similarly, *H.anomala* and *H.aspera* were classified in the same group (Fig. 1). *Hydrangea macrophylla* and *H.serrata* are the most popular species in *Hydrange aceae*. As a result, many cultivars have been developed. The two species had a successful bilateral crossing, and it is presumed that *H. serrata* was widely used to improve the cold hardiness in *H. macrophylla.* For this reason, *H. macrophylla* and *H. serrata* appear to be genetically closer. Consequently, the two species appear to be clustered. Although plants belonging to *H. serrata* are described as having membranous leaves and hairs around the edges and venations (Kim et al. 2016), the *H. serrata* in this study had no hairs on the leaves. It is presumed that the cultivars whose intrinsic characteristics were weakened through horticultural improvements were used in this study.

The reason *H. aspera* and *H. anomala* became one cluster is that there are very few domestically distributed varieties of each species. One cultivar in *H. aspera* and two cultivars in *H. anomala* were used in this study. Exact classification requires experiments with more varieties

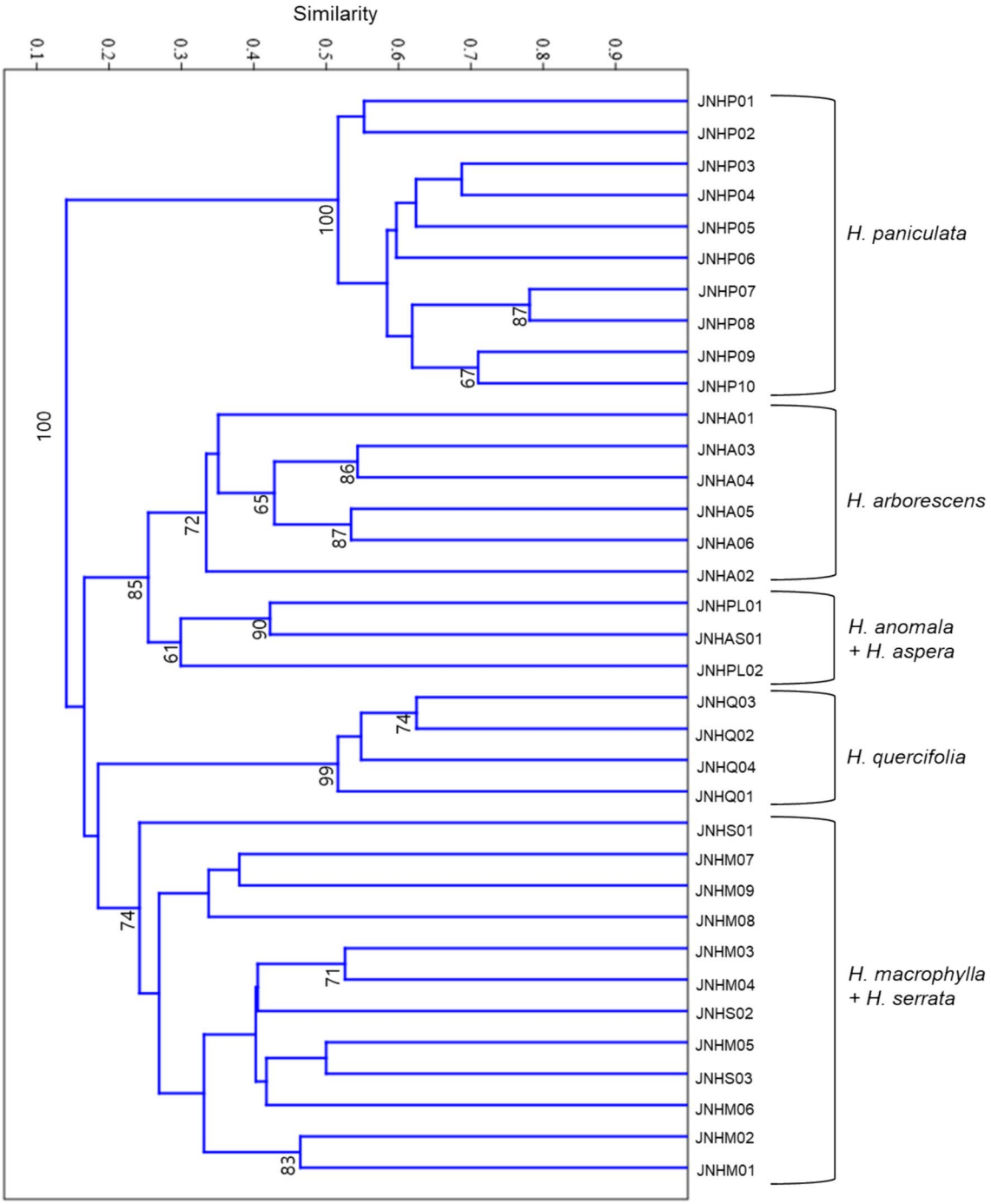

Fig. 1 Unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on the similarities calculated from the simple sequence repeat (SSR) marker data of 35 hydrangea varieties. The bootstrap values (%) out of 1,000 replications are shown when there are more than 60

across two species. Although JNHPL01 in *H. anomala* and JNHAS01 in *H.aspera* were divided into one cluster with a Boot Strap (BS) value of 90%, JNHPL02 in *H. anomala* was divided into a different upper cluster (BS value 56%). JNHPL01 in *H. anomala* was classified as a subspecies (subsp.) or as a separate species (Rinehart et al. 2006; Kim et al. 2016). To objectively identify the genetic relationship, it is necessary to collect the varieties in *H. luteovenosa* and test the SSR markers developed from *H. luteovenosa* in Korea (Ito et al. 2013).

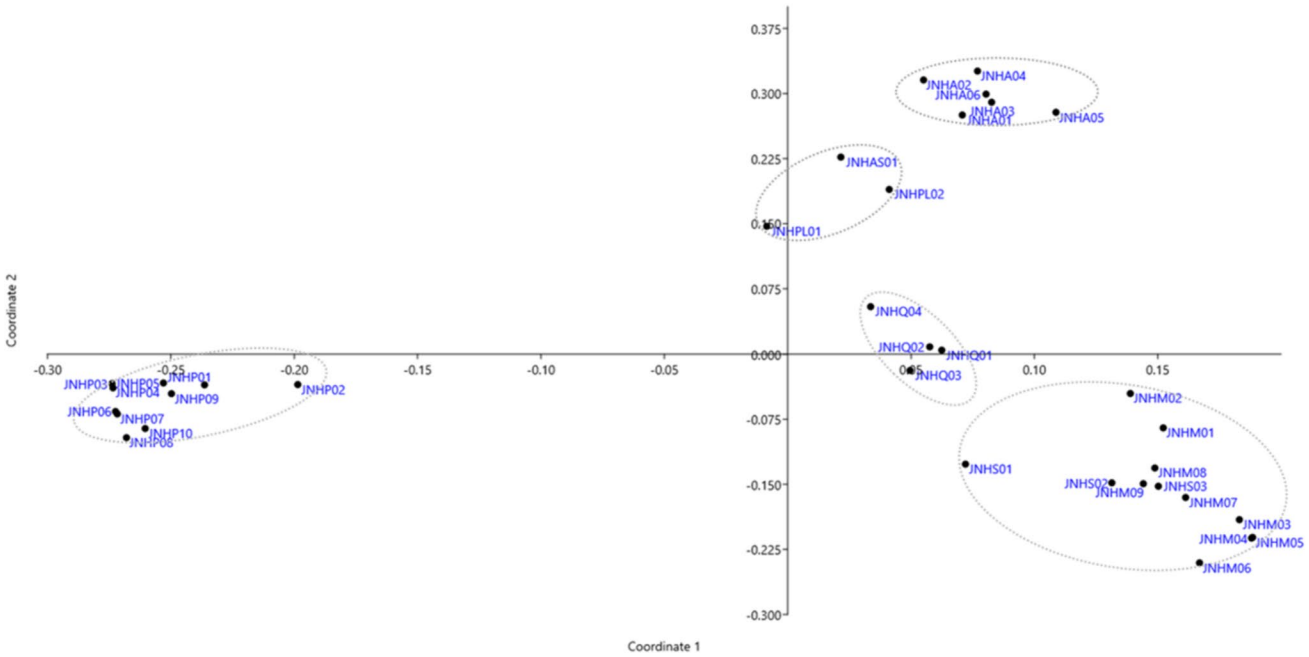

Fig. 2 Two-dimensional (2D) principal coordinate analysis (PCoA) grouping 35 hydrangea genotypes by using the Jaccard similarity coefficient

4.2 Cross compatibility analysis

The history of taxonomic debates between *H. macrophylla* and *H. serrata* has endured for a long time, and studies using molecular techniques have been conducted to resolve them (Wilson 1923; Hara 1955; McClintock 1957; Reed and Rinehart 2007). In this study, *H. macrophylla* and *H. serrata* were grouped into one cluster in the dendrogram, and high cross compatibility between the two species was also confirmed. The produced seeds were able to form many seedlings only by seed sowing without an embryo rescue system. As these results show, there appears to be no reproductive isolation leading to speciation between the two species (*H. macrophylla* and *H. serrata*). Reproductive isolation greatly contributes to speciation and drives its induction inside and outside plants in various ways (Coughlan and Matute 2020). The drives leading to reproductive barriers function more strongly against recently isolated species (Christianson et al. 2005). *Hydrangea macrophylla* and *H. serrata* are rarely reproductive in isolation. In the cases of *H. macrophylla* and *H. serrata*, several drives that produce reproductive isolation between the two species appear to have been weakened or could no longer operate. The taxonomic history of the two species is either quite long, or they have been mixed in the same geographic location for a long time. For this reason, it is presumed that the reproductive isolation and distinction between the two species have been weakened. It is expected to be of practical use in the aspect of cross breeding as a germplasm because the taxonomic debate of *H. macrophylla* and *H. serrata* is meaningless regarding cross breeding. On the other hand, UI was confirmed in the crossing between *H. macrophylla* and *H.paniculata*, but bilateral compatibility between *H. serrata* and *H. paniculata* was confirmed. These results can be used in a breeding program for improving cold hardiness, and it is expected that the progenies of *H. macrophylla* x *H.serrata* can be used for crossing with *H. paniculata*.

Cross-compatibility was analyzed at the species level. Various crosses with varieties are required to confirm UI in the *H. macrophylla* x *H. anomala, H. macrophylla* x *H. quercifolia*, and *H. arborescens* x *H. serrata* combinations. However, clear UI was observed in abundant crosses with diverse varieties between *H. macrophylla* and *H. paniculata*. Previous studies have also mentioned that seed formation is easier when *H. macrophylla* is used as the maternal plant (Cai et al. 2015). It is noteworthy that no seedlings were created by using any species as a maternal plant when *H. macrophylla* was used as a pollen parent, except for when crossed with *H. serrata*. The *H. macrophylla* pollen was thought to be strongly incompatible in the stigma of other species. Meanwhile, *H. serrata*, which is in the same cluster as *H. macrophylla* and has good bilateral cross compatibility with *H. macrophylla*, is able to be crossed with *H. paniculata* and *H.arborescens*. In most crosses, pollen that is of the mob-head type of *H. macrophylla* and the lace-cap type of *H.serrata* were used. However, the pollen seemed to be incompatible according to the inflorescence type. Bak et al. (2021) reported self-incompatibility (SI) in *H. macrophylla*

Female \ Male	*H. macrophylla*	*H. serrata*	*H. arborescens*	*H. paniculata*	*H. quercifolia*	*H. anomala*	*H. aspera*
H. macrophylla		○	△	○	△	△	×
H. serrata	○		×	○	×	×	×
H. arborescnes	△	○		○	△	×	×
H. paniculata	×	△	△		×	×	×
H. quercifolia	×	×	×	×		×	×
H. anomala	×	×	×	×	×		×
H. aspera	×	×	×	×	×	×	

Fig. 3 Cross compatibility of intra- and interspecific hybrids according to the number of progenies through various crosses. ○ means that hybrids produced more than 30 seedlings. △ means that hybrids produced less than 30 seedlings. × means no hybrids

of mob-head type inflorescences and self-compatibility (SC) in *H. macrophylla* of lace-cap type inflorescences. The types of inflorescences seem to be linked to the cross compatibility with *H. macrophylla* and other species. The pollen in *H. serrata* with a lace-cap type inflorescence could be used for crossing. Accordingly, it is expected that the factors affecting cross compatibility are linked to inflorescence genes.

Hydrangea anomala as a pollen plant was able to produce progenies from crossing with *H. macrophylla*, but *H. anomala* as a maternal plant seemed to have strong incompatibility in crossing with other species. In crosses with *H.anomala* as the maternal plant, the JNHPL02 flowers of *H. anomala* died within 1–2 weeks after pollination. Similarly, the JNHPL01 flowers of *H. anomala* died after pollination when crossed with other species. In addition, *H. anomala* was difficult to fertilize even by self-pollination, so incompatibility for crossing was strongly confirmed. However, the pollen germination rate of *H.anomala* was very high, and it has already been confirmed that it can be crossed with *H. macrophylla* (Fig. 3). Accordingly, it is expected that the pollen of *H. anomala* will be useful as a paternal plant. *Hydrangea quercifolia* also had a high pollen germination rate, and it was possible to obtain seedlings by crossing with *H. macrophylla* and *H. arborescens*. *Hydrangea aspera* exhibited male sterility with no pollen and failed to cross with all six species. On the other hand, *H.aspera* was diverse enough to include four subsp., and hybridization with other varieties in the *H.aspera* subsp. was possible (Carespel et al. 2014; Carespel and Morel 2014). In Korea, *H. aspera* is an unpopular species so research is insufficient and collection resources are limited. Thus, more germplasm should be collected, and more experiments should be conducted with *H. aspera*.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13580-024-00629-z.

Acknowledgements This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through (Export Promotion Technology Development Program), funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (315041-05).

Author contribution SH conducted the experiments, data acquisition and manuscript writing. TH participated in data interpretation and revising of the manuscript. All authors have read and agreed to the published version of the manuscript.

Declarations

Conflict of interest Authors declare that they have no conflict of interest.

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