‘Rutpink’ (Scarlet Fire®) Kousa Dogwood

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‘Rutpink’ is a new kousa dogwood (*Cornus kousa*) cultivar released from the woody ornamentals breeding program of the New Jersey Agricultural Experiment Station (NJAES) and Rutgers University and is marketed under the name Scarlet Fire® dogwood (Fig. 1). It exhibits a moderately vigorous, upright growth habit with dark-green foliage and, most notably, large, dark-pink floral bracts (Fig. 2). It has also shown strong field tolerance to the pathogens causing dogwood anthracnose (*Discula destructiva*) and powdery mildew (primarily *Erysiphe pulchra*). Trees of ‘Rutpink’ have exhibited no winter injury under field tests in New Brunswick, NJ, in Zone 7a [0 to 5 °F (USDA Plant Hardiness Zone Map, 2012)]. ‘Rutpink’ was derived from the open pollination of a *C. kousa* seedling (Rutgers K187-44) held in the Rutgers germplasm collection. A parentage analysis using nine single sequence repeat (SSR) markers was conducted to identify its male parent from a pool of 28 potential contributors; results identified the breeding selection Rutgers KN123-6.

Origin

Cornus kousa ‘Rutpink’ is the culmination of a decades-long breeding program at

the NJAES and Rutgers University focused on developing improved big-bracted dogwoods (Molnar and Capik, 2013). It is the first dark-pink bracted kousa dogwood released from the program. While available cultivars of *C. florida*, our native flowering dogwood, express a range of bract colors from white to dark red, bract colors of kousa dogwood (*C. kousa*) are much more limited (Cappiello and Shadow, 2005; Dirr, 2009). Kousa cultivars and seedlings primarily express white bracts, and the few available trees that produce pink bracts are known to be inconsistently colored in the landscape depending on location and year (Cappiello and Shadow, 2005). Furthermore, Trigiano et al. (2004) showed that most of the available pink-bracted *C. kousa*, such as ‘Satomi’, ‘Rosabella’, and ‘Schmred’ (Heartthrob®), are closely related to one another or may even be the same clone with different names. Thus, the phenotypic, and likely genotypic, diversity of pink-bracted kousa cultivars in the landscape is limited.

Despite a lack in diversity of bract colors, kousa dogwoods typically express better adaptation to a wider range of climates, increased disease and pest resistance, and improved stress tolerance when compared with *C. florida* (Augé et al., 2002; Dirr, 2009; Holmes and Hibben, 1989; Li et al., 2009; Mmbaga and Sauvé, 2004; Ranney et al., 1995). Based on this, Dr. Elwin Orton initiated a breeding project in the 1970s at Rutgers University specifically to enhance the bract color and other traits of kousa dogwoods and interspecific hybrids between *C. kousa* and *C. florida* (as well as *C. nuttallii*) with the goal of developing novel, low maintenance yet highly attractive landscape plants (Orton, 1985, personal communication). Our new cultivar stems from a continuation of his breeding efforts. ‘Rutpink’ was derived from an open-pollination event of Rutgers K187-44, a pink-bracted

plant from a cross of *C. kousa* ‘Satomi’ × *C. kousa* ‘Beni Fuji’ made by E. Orton in 1996 (Fig. 3). K187-44 resides in a breeding block in field 70 at the Adelphia Research and Extension Farm, Adelphia, NJ, with 28 other dogwood trees spatially isolated from other dogwoods by at least 2 km (Fig. 4). Flowers of K187-44 were naturally open-pollinated in the spring of 2008, and the seed was harvested in September. This seed was cleaned and stratified, and germinated in the greenhouse in Spring 2009 at Rutgers Horticultural Farm 1, New Brunswick, NJ. After transplanting to larger containers, it was planted into field H3D at the Rutgers Horticultural Farm 3, New Brunswick, NJ, alongside ≈1200 other seedlings of the same age, where it currently resides. From 2009 to 2016, a successive culling approach was used to eliminate poor performing trees from the H3D field and to aid in the identification of superior individuals in the population. Criteria for elimination included the presence of powdery mildew (*E. pulchra*), anthracnose (*D. destructiva*), and/or leaf spot (*Septoria* spp.), poor growth habit (no central leader,

Fig. 1. Original *Cornus kousa* ‘Rutpink’ (Scarlet Fire®) tree in the field at Horticulture Research Farm 3, New Brunswick, NJ.

Fig. 2. Floral bracts of *Cornus kousa* ‘Rutpink’ (Scarlet Fire®) displaying dark-pink color.

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Fig. 3. Parentage of *Cornus kousa* 'Rutpink' (Scarlet Fire®). Date of pollination event is indicated under the male parent.

poor branch structure, and lack of vigor), winter injury to branches and/or vegetative and flower buds, and presence of white or light-pink floral bracts (breeding goal was to obtain primarily dark-pink colored bracts).

The original seedling, located as plant 21 in row 11 of the H3D field, later to be named 'Rutpink', flowered relatively heavily in 2012 (especially when compared with other seedlings in the H3D field), only 4 years after germination. Its precocity (tendency to bloom at a young age) and exceptional dark-pink bract color were first noted that year. Floral bract color ratings were performed from 2012 through 2016 at anthesis, a time when true flowers fully open and bract color is at its peak. 'Rutpink' was by far the most consistently performing plant over the 5-year period in our collection in respect to dark-pink floral bract color and was the only dark-pink bracted plant that expressed other excellent horticultural traits, including an attractive growth habit, freedom from disease, and clean foliage every year since evaluations began. It also had a consistently attractive dark-green leaf color that was absent in many of the other trees in the same field that expressed dark-pink bracts.

Trees of 'Rutpink' were first propagated at Rutgers in 2013. Budwood was sent to nurseries in Tennessee (3) and Oregon (1) for propagation and evaluation in 2014 and 2015. Subsequent testing at the field liner stage (1 year after budding onto seedling rootstocks after standard nursery procedures) has shown the plant to be moderately vigorous to vigorous with very strong branching compared with standard kousa cultivars (A. Neubauer, personal communication). No visible sign of winter injury to vegetative or floral buds has been observed over 9 years for the original tree and 4 years for propagated clones (in New Jersey, Tennessee, and Oregon) under field testing. While 'Rutpink' plants have experienced no insect problems over this period, an inconsequential amount of powdery mildew was observed on the original tree in 2012 and 2014 on sucker growth from the base of the plant on the shaded side and also on late season vigorous growth on a small number of bud-propagated liners in Tennessee in 2014. No powdery mildew was reported in any other years in New Jersey, Tennessee, or Oregon.

Botanical Description

The following descriptions contain color designations according to the Horticultural Color Chart issued by the Royal Horticultural Society of London (1966). 'Rutpink' trees have a densely branched and upright growth habit that forms a rounded but spreading head. Its overall vigor is comparable with the upper range of typical *C. kousa* plants as compared with plants of similar age in its original field of planting in New Brunswick, NJ. At an age of 7 years, the original tree was 3.2 m tall with a 2.5 m spread. The circumference of the crown at 10 cm above the soil level was 0.29 m with mostly smooth textured bark and older stems having some rough parts because of the lenticels. The trunk and older branches are colored 197A to 197B Greyed-Green, with younger stems closer to 200C and 200B Brown. The lenticels on 2 year and older stems are closest to 156B Greyed-White and are typically 1.0–2.0 mm long × 0.3–0.4 mm wide. Similar colored lenticels are also present on younger stems but are smaller, with lengths under 1.0 mm. Branch angles are around 35°–55° for large, major branches, but smaller branches are usually more upright with angles between 45° and 70°.

Because of its tendency to develop many fine branches, foliage is dense across the canopy with ovate/elliptic leaves, acuminate at the tip, and rounded at the base. Leaves are arranged oppositely on stems with four to five veins and moderately wavy margins with some leaves slightly folded toward the adaxial side along the mid vein. The smooth textured adaxial surface is contrasted by the slightly fuzzy abaxial surface caused by tiny trichomes on the blade and tufts of longer, thin hairs in clumps along the midrib, and secondary veins. The leaves are solid colored, with the adaxial surface between 137A and 137B Green and the abaxial side closer to 138B Green. The lower third of the adaxial midvein side changes to 59A Red-Purple, with the same color extending to the lower third of the fourth-fifth secondary veins closest to the petiole. The abaxial midvein is 160D Grey-Yellow, with secondary veins and petioles colored 59A Red-Purple Group. Immature leaves are typically lighter colored, with adaxial surfaces closest to 144A Yellow-Green and interveinal regions between 187A and 187B Greyed-Purple. Veins are the same as on mature leaves, 59A Red-Purple, as are the petioles. The abaxial surface is mostly 146C Yellow-Green with some interveinal areas of 187A Greyed-Purple. In the fall (usually late October–early November in New Jersey), adaxial leaf surfaces lighten to 137A Green, with some random mottling (typical mottling colors include 187A Greyed-Purple Group, 183A Greyed-Purple, 53A Red, 46A Red, 43A Red, and 9A Yellow). Abaxial surfaces are a shade lighter from 138B to 138A.

Inflorescences of 'Rutpink' are single flowers in dense, rounded mounds subtended by showy floral bracts. True flowers per head

range from 38 to 46 with an average of 42 ($n = 20$). Each flower has four stamens, with filaments that are roughly 2.3 mm long by 0.3 mm wide and are colored 155B White. Anthers are about 0.4 mm long and 0.8 mm wide, colored 162A Greyed-Yellow. The style is around 1.5 mm long by 0.3 mm wide and is closest to 144C Yellow-Green. The stigma is the same color as the style and measures 0.3 mm tall by 0.5 mm wide (for reproductive measurements, $n = 4$ and numbers are an average of all measurements). Pollen is 144C Yellow-Green. Flowers are not fragrant or persistent, whereas the floral bracts last around 4 weeks or more depending on weather and other factors. Peduncles range from 63 to 110 mm long (average 79 mm). Anthesis typically occurs in late May in New Jersey (May 23–28).

The mounded flower head is encircled by two sets of floral bracts (two upper and two lower), which reach their peak size about the time of anthesis or a few days after. The bracts are distinctly acuminate with apices ending in long, thin points with obtuse to rounded bases. In typical flower heads, bracts barely overlap at anthesis (less than 10% of total width). Each bract is generally about the same size on a flower head, with the lower set being slightly larger. Upper bracts average 66.4 mm long (60–75 mm range) and 31.9 mm wide (26–45 mm range), with a spread across the bracts of 133.6 mm (122–150 mm range) ($n = 50$ for all bract measurements). Lower bracts have an average length of 67.3 mm (61–75 mm range) and width of 35.2 mm (31–41 mm range), with a spread of 134.5 mm (123–148 mm range). Bract size varies slightly from year-to-year depending on conditions such as environment and total number of flower heads per branch (very dense flower head numbers tend to result in smaller bract size), but the bract size relationship between upper and lower bracts remains constant (data not shown). At the pinnacle of bract color display (usually between the last week of May and the first week of June), adaxial bract surfaces are between 54A and 54B Red tending toward 54B. Abaxial surfaces are closer to 51C Red with some veins colored 51B Red. The flower head peduncle is 144C Yellow-Green. Several days after peak color is reached, bracts lighten to 55D Red before senescence.

'Rutpink' flower heads produce two-celled, usually one-seeded fleshy drupes that mold into a mounded syncarp that resembles a large blackberry or raspberry, which is typical for *C. kousa*. This nearly round aggregate fruit averages 23.2 mm long, 22.3 mm wide, and 21.0 mm high and is mostly smooth except for the dried vestigial floral parts (sepals, style, and stigma) found at the apex of individual drupes. The fruit varies in color as it reaches maturity. Color transitions from green to yellow to orange to pink and reaches 46A–42C Red at maturity in late August. They will typically persist on the tree for up to 6 weeks after reaching maturity. The seeds are small and bony, averaging 6.4 mm long × 4.8 mm wide × 3.6 mm deep

Fig. 4. Spatial relationships between potential male parents and female parent K187-44 in Field 70 at the Adelpia Research and Extension Farm, Adelpia, NJ. Each box on the grid represents a distance of 8.0 ft (2.44 m). The field was originally planted in its entirety with trees planted on a spacing of 8.0 ft (2.44 m) $\times$ 8.0 ft (2.44 m). All trees have been removed except those present on the diagram. Row numbers are listed along the bottom and top and tree number within the row on the left side of the figure. The female and the identified male parent are indicated by red color boxes and one and two asterisks, respectively.

($n = 40$) and are colored 165C–165D Greyed-Orange, and shaped like those of typical *C. kousa*. They average 16 seed per fruit ($n = 36$).

Parentage Analysis

As previously stated, ‘Rutpink’ was derived from an open-pollination event; the

male parent was unknown at the time of seed collection. Fortunately, the mother tree K187-44 is located in an isolated breeding block with only 28 potential pollen contributors (Fig. 4). The pool of potential male parents includes a wide variety of select breeding accession comprising *C. kousa* as well as advanced generation *C. kousa* $\times$ *C. nuttallii* and *C. kousa* $\times$ *C. florida* hybrids. Newly emerged leaf tissue was collected from ‘Rutpink’, its female parent K187-44, and all 28 possible male parents located at the Rutgers Adelpia Research farm in Spring 2015. Seven additional accessions were also included in the study to examine their genetic relationships to the new cultivar, which included two half-siblings of ‘Rutpink’ (same mother K187-44), two well-known pink *C. kousa* cultivars (Satomi and Rosea), one *C. kousa* accession used widely in the Rutgers breeding program (*C. kousa* K2), and two pink-bracted F_1 *C. kousa* $\times$ *C. florida* hybrids [*Cornus $\times$ rutgersensis* ‘Rutgan’ (Stellar Pink®) and its full sibling Rutgers KF45-29].

The tissue was flash frozen in liquid nitrogen soon after collection and ground with a mortar and pestle. DNA extractions were performed using a modified CTAB protocol adapted from Cullings (1992) and Doyle and Doyle (1987). Extracted DNA was quantified with a spectrophotometer (NanoDrop ND-1000; Thermo Scientific, Waltham, MA) and diluted to a concentration of 5 ng- μ L⁻¹. Nine SSR markers (Table 1) were used to genotype all 37 accessions; five were developed from the *C. florida* genome (Wang et al., 2008) and four were developed from the *C. kousa* genome (Wadl et al., 2008). The M13 (–21) 18-bp sequence was added to the 5’ end of all forward primers as a cost effective means of fluorescent labeling of polymerase chain reaction (PCR) fragments (Schuelke, 2000), and the “PIG-tailing” sequence (GTTTCTT) was included at the 5’ end of all reverse primers to reduce uncertainty in scoring “true” vs. “plus-A” alleles (Brownstein et al., 1996). Primers were synthesized by Integrated DNA Technologies (Coralville, IA). PCR amplifications were performed in 96 well plates in 13- μ L reaction volumes. Reactions consisted of $\approx$ 5 ng genomic DNA, 10xRamp-Taq PCR buffer (Denville Scientific, Metuchen, NJ), 2.0 mM MgCl₂, 0.25 mM each dNTP (Denville Scientific), 0.5 U Ramp-Taq DNA polymerase (Denville Scientific), 0.5 pmol forward primer with 5’ M13 (–21) addition, 1.0 pmol reverse primer with 5’ PIG-tail addition, and 1.0 pmol FAM, NED, PET, or VIC fluorescently labeled M13 (–21) primer. PCR cycling was performed in GeneAmp 9700 thermocyclers (Applied Biosystems, Foster City, CA) using the following parameters: initial denaturation of 94 °C for 5 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 45 s, followed by 20 cycles of 94 °C for 30 s, 53 °C for 45 s, and 72 °C for 45 s, followed by a final extension of 72 °C for 10 min. PCR products were run on a capillary electrophoresis genetic analyzer (ABI 3500xl; Applied Biosystems) and

Table 1. Simple sequence repeat (SSR) loci used for parentage and genetic diversity analyses of *Cornus kousa* ‘Rutpink’ (Scarlet Fire®) and its potential *Cornus* parental accessions.

Marker	Sequence	Repeat motif	Expected amplicon size (bp)	Reference
² CF_47	F: CCTGTCCTGAAATTGTTCAACAGTAG R: GTTGAATTATGCATGCTATCTTGAATTT	(AC) ₁₅	121	Wang et al. (2008)
CF_048	F: GCTTTGACATCCTCTTTGCTTCTC R: AAGAGGCTTCACAAGACAATCAGC	(TG) ₉	144	Wang et al. (2008)
CF_055	F: TGGAGTAGGGCAAAAGATCAAGAG R: TCCAGGGAATGTTTCGGTAGATTAG	(GT) _{7T} (TG) ₁₀	155	Wang et al. (2008)
CF_125	F: GGTGAATTGAAGATTGATTGTTTCA R: GGAATTAAGGCGAGAATGATGATG	(AC) ₁₀	155	Wang et al. (2008)
CF_597	F: AAGTCAGATCATTTCAGATTAACA R: CGAATTGACGATAAAATACAAAATA	(AC) ₁₃	107	Wang et al. (2008)
³ CK_015	F: GTCAAATTTTGTCTTTCTCTCT R: GGAGAGACAGAGTACAGTAGAGGT	(CT) ₁₀	103–136	Wadl et al. (2008)
CK_031	F: TGTCAGTCTTACAGAAACAAT R: TATGACGAGATTGTATAAGTTGCT	(CT) ₇	114–138	Wadl et al. (2008)
CK_040	F: CCAAGTCAGTTGGTAGTAATTC R: AGTGCAACTTTACTTGCTATGT	(GT) ₁₆	80–114	Wadl et al. (2008)
CK_092	F: TACTGTGAGAAAXAAAACAAAATG R: TCATTCTTAACCTTTTACCTCAA	(GA) _{8T} (AG) ₁₃	105–138	Wadl, unpublished

²CF denotes *Cornus florida* derived SSR markers.

³CK denotes *Cornus kousa* derived SSR markers.

Table 2. Summary statistics for each simple sequence repeat locus and the average value across all loci for *Cornus kousa* ‘Rutpink’ (Scarlet Fire®) and its potential *Cornus* parental accessions.

Locus	Alleles (no.)	Ho ²	He ³	PIC ⁴	F (null) ⁵
CK_040	11	0.207	0.891	0.863	0.6208
CK_031	4	0.467	0.725	0.663	0.217
CF_047	7	0.167	0.676	0.627	0.5945
CF_048	4	0.367	0.588	0.504	0.2171
CF_055	4	0.433	0.375	0.346	–0.1105
CK_015	15	0.867	0.872	0.846	–0.0047
CK_092	11	0.966	0.886	0.857	–0.0559
CF_125	6	0.31	0.62	0.576	0.317
CF_597	13	0.7	0.901	0.875	0.1172
Mean	8	0.498	0.726	0.6841	—

²Observed heterozygosity.

³Expected heterozygosity.

⁴Polymorphism information content.

⁵Estimated null allele frequency.

Fig. 5. Parents of *Cornus kousa* ‘Rutpink’ (Scarlet Fire®) dogwood. Upper left, K187-44, female parent; upper right, KN123-6, identified male parent; and below, ‘Rutpink’. Note the light pink color and similar bract shapes and sizes of each parent to ‘Rutpink’. Photo not to scale.

sized using a LIZ600 size standard (Applied Biosystems). The GeneScan Installation Standard DS-33 (Applied Biosystems) was added to the last well of each plate run.

SSR marker genotyping results were analyzed and binned using Genemapper 4.0 (Applied Biosystems). SSR marker data were

then compiled and exported into Microsoft Excel, and an allele frequency analysis was performed using CERVUS 3.0 to ensure the loci were suitable for parentage analysis (Kalinowski et al., 2007). The following allele frequency parameters were used to assess each loci: the number of alleles per locus [allele (no.)], observed heterozygosity (Ho), expected heterozygosity (He), polymorphism information content, and the frequency of null alleles [F(Null)] (Table 2). A parent pair simulation analysis was then run using CERVUS with parameters, including sexes unknown and with 100,000 offspring to determine the critical threshold log-likelihood statistics [logarithm of the odds (LOD) score] where a true parent could be distinguished from an unrelated candidate parent. If the LOD score for a potential parent exceeded the critical threshold run, it was considered statistically significant at the 95% level and thus was likely the parent. Note that the seven additional accessions described previously, which were not in the pool of potential male parents, were not included in the Cervus parental analysis.

To assess genetic relationships among all 37 accessions, allelic data were inputted into Powermarker to create a frequency based distance matrix (Liu and Muse, 2005). An

unweighted pair group method using arithmetic averages (UPGMA) dendrogram was constructed from the distance matrix and visualized in Mega 5.01 (Tamura et al., 2011). Bootstrap values were calculated and the program CONSENSE, a component of the program package PHYLIP version 3.695 (Felsenstein, 1989), was used to create a consensus tree with a minimum support value of 0.500.

Results of Parentage and Diversity Analysis

Parentage analysis identified the breeding selection KN123-6 as the most likely male parent (95% confidence level). KN123-6 had a pair LOD score of 0.038 and the mother (K187-44), father, and offspring (‘Rutpink’) together had a trio LOD score of –1.55. These findings are reinforced by the relatively close proximity of the plants in the field (≈30 m; Fig. 4) and their complimentary phenotypes (Fig. 5). Both are light-pink bracted plants that express pure kousa phenotypes and overlapping dates of anthesis; K187-44 is of pure kousa background and KN123-6 is largely kousa with a small contribution from *C. nuttallii* (Fig. 3), but it resembles pure kousa. Previous research and unpublished data have revealed that red/pink bract color is likely controlled at a single locus and is recessive to white color (Orton, 1982, personal communication). Thus, it is reasonable that some of the offspring of K187-44 and KN123-6 expressed pink bracts. However, when compared with its parents, the enhanced color of ‘Rutpink’ cannot be fully explained by our previous work of crossing light pink bracted kousas, which typically yields light pink offspring. The identified male parent KN123-6 has several unknown parents in its pedigree and could carry an unknown allele or modifying factors that contributed to the enhanced color. Thus, further work is needed to clarify the genetic control of pink bracts in *C. kousa*.

Also reinforcing the parentage results, the UPGMA dendrogram (Fig. 6) places

Fig. 6. Genetic relationships of *Cornus kousa* 'Rutpink' (Scarlet Fire®), its female parent K187-44, the 28 potential male parents, and seven other genotypes through an unweighted pair group method using arithmetic averages dendrogram. Branches with bootstrap support of >50% are labeled.

'Rutpink' in a cluster with its identified male parent KN123-6, KN123-12 (a sibling of the identified male), and two of its siblings arising from open pollination from the same harvest date (H3DR11P24 and H3DR11P29). The dendrogram also shows that 'Rutpink' is distinct from its maternal grandparent *C. kousa* 'Satomi', placing it in adjacent clade to the one described previously. On a side note, the results also support previous work suggesting that 'Satomi' is genetically similar to other available pink kousa dogwoods (Trigiano et al., 2004), as 'Satomi' and 'Rosea' have nearly identical allele profiles. Furthermore, the UPGMA placement of 'Rutpink' shows that it is distinct from and relatively distantly related to the series of hybrid

dogwoods previously released from Rutgers University (Hyperion®, Stellar Pink®, Aurora®, Constellation®, Venus®, and Ruth Ellen®), all of which (besides Venus®) share a common lineage (*C. kousa* K2). This establishes 'Rutpink' as a new cultivar that adds to genetic diversity in the landscape beyond what has been released from Rutgers previously.

Availability

'Rutpink' has been shown to develop strong, well-branched plants from traditional chip budding onto field grown seedling rootstocks of *C. florida* and *C. kousa*. It has also been successfully propagated and rooted through plant tissue culture, although no attempts have been made yet on a commercial scale. A U.S. plant patent is pending (US2017/0055409P1) and Plant Breeders rights have been applied for in the European Union. It is licensed for propagation and sale by several U.S. nurseries, a list of which can be found at http://agproducts.rutgers.edu/dogwood/scarlet_fire.html or obtained by contacting the Rutgers University Office of Research Commercialization, 33 Knightsbridge Road, second Floor East, Piscataway, NJ 08854.

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