

Genetic characterisation of the petal spot phenotype for *Gossypium arboreum* accession PI 408798

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Abstract: The occurrence of floral petal spots is common among flowering plants and plays a major role in attracting pollinators. Cotton genotypes having large red petal spots are frequent in the United States Department of Agriculture, National Plant Germplasm System *Gossypium arboreum* (L.) collection. One accession, PI 408798, showed a unique faint red petal spot phenotype. To genetically characterise this trait, a F_2 population of 226 plants was developed by crossing PI 408798 with *G. arboreum* accession PI 529714 that lacked pigmented petal spots. From the population, 161 plants showed the presence of faint red petal spots; whereas, 65 plants lacked pigmented petal spots. These data supported the single dominant gene model for the presence of floral petal spots. Accession PI 408798 will provide an important genetic resource to characterise the genes involved in the pathway controlling floral pigmentation.

Keywords: anthocyanin; Asiatic cotton; floral pigmentation; germplasm

Petal spots typically occur as distinctly pigmented regions at the base of flower petals. The development of petal spots has been widely observed in angiosperm species including eudicot and monocot plants (Thomas et al. 2009). Petal spots are considered to function in the attraction of pollinators and may have coevolved to attract specific pollinator species (Johnson & Dafni 1998). The evolution of petal spots in angiosperm species has resulted in a wide range of phenotypic and functional diversity in colour, texture, scent or nectar production, heat trapping capability, UV light reflectance, tactile surface to aid in navigating the corolla, and patterns mimicking the pollinator species (Johnson & Midgley 1997; Thomas et al. 2009). Petal spots are also an economically important trait for the horticultural and cut flower industries.

The majority of the more than 50 cotton species in the genus *Gossypium* show the formation of pigmented petal spots. Cotton is mainly cultivated to produce fibre for the textile industry. Four *Gossypium* species, *G. hirsutum* (L.), *G. barbadense* (L.),

G. arboreum (L.), and *G. herbaceum* (L.), are cultivated for the production of spinnable fibre (Ma et al. 2008; Zhu et al. 2019). *Gossypium hirsutum* and *G. barbadense* are allotetraploid species originating in the Americas and referred to as New World cottons (Wendel 1989). *Gossypium hirsutum* (upland cotton) is the most widely cultivated species representing more than 95% of worldwide production; whereas, *G. barbadense* represents approximately 2% of worldwide production and is cultivated for its high-quality fibre (Zhang et al. 2008; Zhu et al. 2019). *Gossypium arboreum* and *G. herbaceum* are diploid species and referred to as Asiatic or Old World cottons with *G. arboreum* representing approximately 2% of worldwide production (Wendel 1989; Guo et al. 2006; Zhu et al. 2019). Because of low fibre yields and fibre quality, Asiatic cottons are not widely cultivated and are mainly cultivated under low input production systems in regions of Asia that are not suitable for the production of *G. hirsutum* cultivars (Guo et al. 2006; Iqbal et al. 2015). Asiatic cottons however are an important source of genes

for stress tolerance, disease and pest resistance, and a source of genetic diversity for fibre yield, seed production, and fibre quality traits (Mehetre et al. 2003; Ma et al. 2008; Maqbool et al. 2010; Erpelding & Stetina 2018). Additionally, the diploid genome makes it easier to study key genetic and regulatory mechanisms for specific traits and this knowledge can be transferred to allotetraploid species for cotton improvement.

Modern *G. hirsutum* cultivars lack pigmented petal spots; however, a wide range of phenotypic diversity is present in primitive races (Harland 1929; Stephens 1974; Wilson 1987; Zumba & Myers 2008). Phenotypic diversity for petal spots is also present in *G. barbadense* germplasm accessions (Stephens 1974; Zumba & Myers 2008). These species showed a diverse range in colour intensity for petal spot development (Harland 1929). In contrast, diversity is lacking for *G. arboreum* genotypes with only a slight variation in colour intensity reported for petal spot phenotypes (Harland 1929; Hutchinson 1932; Stephens 1948). The R_2 gene associated with the A-genome of cotton was reported to confer expression of the petal spot phenotype (Hutchinson 1932; Stephens 1955). Wilson (1987) reported that the R_2 gene showed incomplete dominance and was affected by modifiers and the environment for *G. hirsutum* genotypes. The majority of the genetic studies for *G. arboreum* and *G. herbaceum* genotypes reported multiple alleles for the petal spot gene with as many as 20 alleles (Hutchinson 1932; Silow & Yu 1942; Stephens 1948; Yu & Chang 1948); however, these studies also considered that pigmentation of stems and leaves was conferred by the same gene. Yu and Chang (1948) proposed that three closely linked genes near the centromere conferred the petal spot and plant pigmentation phenotypes with recombination occurring at a low frequency. In contrast, vegetative pigmentation for *G. hirsutum* and *G. barbadense* genotypes is conferred by the R_1 gene located on the D-subgenome (Stephens 1955). Pigmentation of floral petal spots involves the anthocyanin pathway (Stephens 1948; Zhang et al. 2015) and understanding the genetics of petal spot development will aid in determining the regulatory mechanisms of this pathway.

Gossypium arboreum accessions from the United States Department of Agriculture (USDA), National Plant Germplasm System (NPGS) cotton collection (accessible under Plant Germplasm, at www.ars-grin.gov) typically show the dark red petal spot phenotype and pigmented plants. Accessions without petal spots

are also present in the collection and the majority of these accessions typically show green non-pigmented stems and leaves. Accession PI 408798 from this collection showed a rare petal spot phenotype. This accession showed the green non-pigment stem phenotype typical of genotypes lacking pigmented petal spots; however, flowers showed faint red petal spots. The aim of this study was to characterise the genetics of this unique petal spot phenotype. A segregating F_2 population was developed for petal spot evaluation by crossing PI 408798 with *G. arboreum* accession PI 529714 showing the absence of petal spots. This population allows for the identification of genes associated with pigment development and could be useful for crop improvement as anthocyanins have diverse functions in plants.

MATERIAL AND METHODS

Gossypium arboreum accession PI 408798 (inventory ID A₂-260) was donated to the NPGS cotton collection in 1976 (<https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1308341>) and was originally collected from China. The atypical petal spot phenotype of PI 408798 was identified during a seed increase of accessions from the NPGS *G. arboreum* collection. The accession showed faintly red coloured petal spots compared to the dark red petal spots typically observed in *G. arboreum* accessions (Figure 1). Accession PI 529714 (inventory ID A₂-11) was donated to the NPGS collection in 1989 (<https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1424650>) and lacks the pigmented petal spot phenotype. Flowers of PI 408798 and PI 529714 were self-pollinated and single plant selections from self-pollinated fruits were conducted over two generations in the field at the USDA in Stoneville, Mississippi for seed purification. To establish a segregating population, PI 408798 was used as the female parent and PI 529714 as the male parent in the cross (Erpelding et al. 2014). Seeds from the single plant selections were planted in 3.05 m plots in Stoneville and phenotypically evaluated to assess uniformity of parental genotypes prior to crossing. Twenty delinted seeds derived from one cross were planted in a single 3.05 m row in the field at Stoneville and flowers on F_1 plants were self-pollinated by covering flower buds with 10 × 15 cm organza mesh bags (Uline, Pleasant Prairie, USA). The seed cotton was removed from self-pollinated fruits, ginned, and delinted. For the F_2 population, 25 seeds were planted

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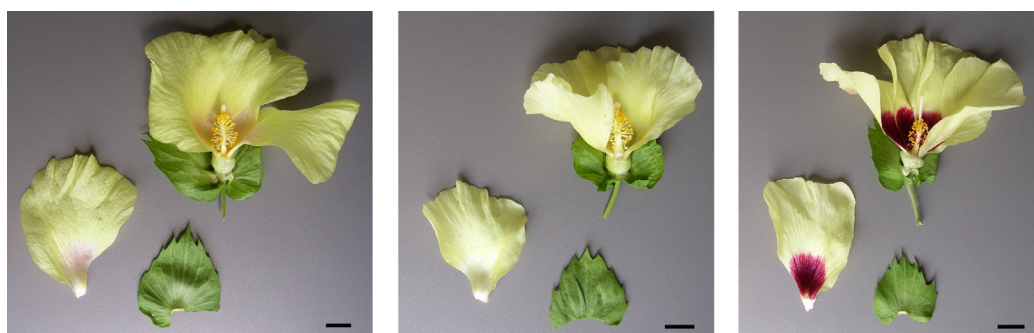


Figure 1. Flowers for *Gossypium arboreum* accessions PI 408798 (left) showing the faint red petal spots and PI 529714 (centre) showing the absence of pigmented petal spots; these two accessions were used as parents to develop the F₂ population; accession PI 615699 (right) showing red petal spots is an example of the most frequent petal spot phenotype observed for *G. arboreum* genotypes
Black bars represent a 1 cm scale

in 3.05 m rows with a total of 35 rows planted. The parental accessions were also planted in single 3.05 m rows for phenotypic comparison with F₂ plants. The standardised cotton descriptors were used for phenotypic evaluations (https://www.cottongen.org/data/trait/NCGC_rating_scale). Individual plants from the population were tagged and flowers were characterised for the presence or absence of faint red petal spots (Figure 1). The segregation data for the petal spot phenotypes were tested using the Chi-square test of significance with the Statistics 9 software package (Analytical Software, Tallahassee, USA).

RESULTS

The characteristic phenotypes for the parental accessions PI 408798 and PI 529714 are presented in

Figure 2. The accessions have green non-pigmented stems that are pubescent, broad lacinate shaped leaves with five major lobes, and flowers with a yellow coloured corolla and dark yellow pollen. Leaves and flowers of PI 408798 are generally larger than PI 529714. The accessions also differ for growth habit. PI 408798 tends to be shorter in height with the production of longer lateral branches. This branching characteristic results in a more prostrate growth habit with plant lodging and stem breakage observed under field conditions in Stoneville. PI 529714 shows an upright growth habit with plant heights ranging from 1 to 1.5 m. Lateral branches are typically shorter in PI 529714 and flowers tend to be more clustered on these branches. PI 408798 is later in maturity with flower initiation approximately 10 days later than PI 529714. Additionally, PI 408798 typically



Figure 2. Leaf shape, stem colour, flower corolla colour, and petal spot phenotypes for *Gossypium arboreum* accessions PI 408798 (left) and PI 529714 (right) used as parents to determine the inheritance of the faint red petal spot trait for PI 408798
Black bars represent a 1 cm scale

Table 1. Flower petal spot segregation data for 226 F₂ plants derived from the *Gossypium arboreum* cross PI 408798 × PI 529714 used to determine the inheritance of the faint red petal spot phenotype observed for accession PI 408798; accession PI 529714 shows the absence of pigmented petal spots

Petal spot	No. of plants	χ^2 (3 : 1 segregation ratio)	P-value
Present	161	1.705	0.1916
Absent	65		

produces few flowers and fruits. Fruits are similar in shape for the accessions and are typical of *G. arboreum* genotypes.

Flowers for F₁ plants derived from the cross PI 408798 × PI 529714 showed the faint red petal spot phenotype characteristic of the PI 408798 parent suggesting the trait is dominant. The segregation data for the F₂ population are presented in Table 1. Excessive rainfall after planting resulted in a low seed germination rate for the population thus data were collected for 226 plants. The majority of plants showed the faint red petal spot phenotype and the population data supported the single gene model with the presence of the PI 408798 petal spot phenotype dominant over the absence of pigmented petal spots. The colour intensity of the petal spots observed for the F₂ plants was similar to the PI 408798 parent; although some slight variation in colour intensity was observed, but this variation may have resulted from differences in flower age at the time of the evaluation. The F₂ plants also tended to show leaves similar in size to the PI 529714 parent while the size of the flowers was more similar to the PI 408798 parent. The majority of the plants showed a more upright growth habit, but lodging was observed for nearly all plants. The F₂ plants were generally taller than the parents with heights ranging from 1.5 to 1.8 m. The length of lateral branches was intermediate between the parents. Green non-pigmented stems and leaves were observed for all plants in the population.

DISCUSSION

More than 1 600 accessions are conserved in the NPGS *G. arboreum* collection (accessible under Plant Germplasm, at www.ars-grin.gov); however, not all accessions have been phenotypically characterised under field conditions in Stoneville. Nonetheless, PI 408798 was the only genotype that showed the

faint red petal spot phenotype from more than 500 accessions evaluated in the collection. Accessions with dark red petal spots were more frequently observed and these accessions also showed red or sun-red pigmented stems. These results coincide with previous research showing red petal spots for flowers of *G. arboreum* genotypes were the most frequently reported phenotype (Hutchinson 1932; Silow & Yu 1942; Yu & Chang 1948). Less than 5% of the accessions included in the seed increase showed no petal spots. Except for PI 408798, all accessions with green non-pigmented stems and leaves showed no petal spots. The dark red petal spot phenotype was shown to be conferred by a single dominant gene (Erpelding 2017). Similarly, the faint red petal spot phenotype observed for PI 408798 in the present study was conferred by a single dominant gene. These data are supported by previous research in which the presence of pigmented petal spots was dominant over non-pigmented petal spots (Hutchinson 1932; Silow & Yu 1942; Yu & Chang 1948; Stephens 1974).

Two phenotypes for the absence of floral petal spots in *G. arboreum* genotypes have been reported by Hutchinson (1932). One phenotype was characterised by uniformly coloured petals and referred to as spotless. The gene symbol *r*^o was proposed for this spotless phenotype (Hutchinson 1932). The second phenotype showed a white non-pigmented spot at the base of the petal where the petal spot would typically occur and was referred to as ghost spots (Harland 1929). This phenotype could only be observed for flowers with a pigmented corolla and the ghost spots covered a similar region of the petals as observed for accessions with petal spots. The gene symbol *r*^g was proposed for this ghost spot phenotype (Hutchinson 1932). The parental accession PI 529714 used in the present study showed the ghost spot phenotype. In crosses between *G. herbaceum* and *G. arboreum* genotypes, Hutchinson (1932) identified a unique petal spot phenotype, which was similar to the petal spot phenotype observed for PI 408798 in the present study. This phenotype showed ghost spots at the base of the petals with smaller pigmented spots observed in the center of these ghost spots surround by a white non-pigmented margin. Accession PI 408798 was originally classified as *G. herbaceum* when donated to the NPGS collection. Although PI 408798 is more characteristic of *G. arboreum* genotypes, it is possible that the petal spot trait resulted from a hybridization between *G. herbaceum* and *G. arboreum* genotypes, which led to the development of PI 408798.

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Hutchinson (1932) however reported that a recessive gene conferred this phenotype and that the phenotype never bred true. Crossing PI 408798 with the red petal spot and spotless phenotypes to develop segregating populations would be useful to further evaluate the genetics of petal spot development in *G. arboreum*. In addition to petal spot and vegetative pigmentation, anthocyanins have an important role in plant defense; thus, characterising the genetics of anthocyanin biosynthesis is essential to understand the regulatory mechanisms of this pathway.

REFERENCES

- Erpelding J.E. (2017): Inheritance of the ovule fuzzless trait for *Gossypium arboreum* germplasm line PI 529708. *International Journal of Plant Breeding and Genetics*, 11: 25–30.
- Erpelding J.E., Stetina S.R. (2018): Genetic characterization of reniform nematode resistance for *Gossypium arboreum* accession PI 417895. *Plant Breeding*, 137: 81–88.
- Erpelding J.E., Stetina S.R., Turley R.B. (2014): Successful boll development after ovary damage during emasculation of upland cotton flowers. *Journal of Plant Breeding and Crop Science*, 6: 73–76.
- Guo W.Z., Zhou B.L., Yang L.M., Wang W., Zhang T.Z. (2006): Genetic diversity of landraces in *Gossypium arboreum* L. race *sinense* assessed with simple sequence repeat markers. *Journal of Integrative Plant Biology*, 48: 1008–1017.
- Harland S.C. (1929): The genetics of cotton. Part I. The inheritance of petal spot in new world cottons. *Journal of Genetics*, 20: 365–385.
- Hutchinson J.B. (1932): The genetics of cotton. Part VIII. The inheritance of anthocyanin pigmentation in Asiatic cottons. *Journal of Genetics*, 26: 317–339.
- Iqbal M.A., Abbas A., Zafar Y., Rahman M.U. (2015): Characterization of indigenous *Gossypium arboreum* L. genotypes for various fiber quality traits. *Pakistan Journal of Botany*, 47: 2347–2354.
- Johnson S.D., Midgley J.J. (1997): Fly pollination of *Gorteria diffusa* (Asteraceae), and a possible mimetic function for dark spots on the capitulum. *American Journal of Botany*, 84: 429–436.
- Johnson S.D., Dafni A. (1998): Response of bee-flies to the shape and pattern of model flowers: implications for floral evolution in a Mediterranean herb. *Functional Ecology*, 12: 289–297.
- Ma X.X., Zhou B.L., Lü Y.H., Guo W.Z., Zhang T.Z. (2008): Simple sequence repeat genetic linkage maps of A-genome diploid cotton (*Gossypium arboreum*). *Journal of Integrative Plant Biology*, 50: 491–502.
- Maqbool A., Abbas W., Rao A.Q., Irfan M., Zahur M., Bakhsh A., Riazuddin S., Husnain T. (2010): *Gossypium arboreum* GHSP26 enhances drought tolerance in *Gossypium hirsutum*. *Biotechnology Progress*, 26: 21–25.
- Mehetre S.S., Aher A.R., Gawande V.L., Patil V.R., Mokate A.S. (2003): Induced polyploidy in *Gossypium*: a tool to overcome interspecific incompatibility of cultivated tetraploid and diploid cottons. *Current Science*, 84: 1510–1512.
- Silow R.A., Yu C.P. (1942): Anthocyanin pattern in Asiatic cottons. *Journal of Genetics*, 43: 249–284.
- Stephens S.G. (1948): A biochemical basis for the pseudo-allelic anthocyanin series in *Gossypium*. *Genetics*, 33: 191–214.
- Stephens S.G. (1955): Linkage in upland cotton. *Genetics*, 40: 903–917.
- Stephens S.G. (1974): Geographic and taxonomic distribution of anthocyanin genes in new world cottons. *Journal of Genetics*, 61: 128–141.
- Thomas M.M., Rudall P.J., Ellis A.G., Savolainen V., Glover B.J. (2009): Development of a complex floral trait: the pollinator-attracting petal spots of the beetle daisy, *Gorteria diffusa* (Asteraceae). *American Journal of Botany*, 96: 2184–2196.
- Wendel J.F. (1989): New world tetraploid cottons contain old world cytoplasm. *Proceeding of the National Academy of Sciences USA*, 86: 4132–4136.
- Wilson F.D. (1987): Inheritance of pink filament in cotton. *Journal of Heredity*, 78: 223–224.
- Yu C.P., Chang T.S. (1948): Further studies on the inheritance of anthocyanin pigmentation in Asiatic cotton. *Journal of Genetics*, 49: 46–56.
- Zhang H-B., Li Y., Wang B., Chee P.W. (2008): Recent advances in cotton genomics. *International Journal of Plant Genomics*, 2008: Article ID 742304 20p.
- Zhang Y., Cheng Y., Ya H., Xu S., Han J. (2015): Transcriptome sequencing of purple petal spot region in tree peony reveals differentially expressed anthocyanin structural genes. *Frontiers in Plant Science*, 6: 964.
- Zhu L., Tyagi P., Kaur B., Kuraparthi V. (2019): Genetic diversity and population structure in elite U.S. and race stock accessions of upland cotton (*Gossypium hirsutum*). *Journal of Cotton Science*, 23: 38–47.
- Zumba J.X., Myers G.O. (2008): Evaluation of the USDA Shafter cotton (*Gossypium* spp.) collection for agronomic and fiber traits. *Journal of Cotton Science*, 12: 73–80.

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