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Invited Review

Buckwheat heteromorphic self-incompatibility: genetics, genomics and application to breeding

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Common buckwheat (*Fagopyrum esculentum* Moench $2n = 2x = 16$) is an outcrossing crop with heteromorphic self-incompatibility due to its distylous flowers, called pin and thrum. In pin plants, a long style is combined with short stamens and small pollen grains; in thrum plants, a short style is combined with long stamens and large pollen grains. Both the intra-morph self-incompatibility and flower morphology are controlled by a single genetic locus named the *S* locus; thrum plants are heterozygous (*Ss*) and pin plants are homozygous recessive (*ss*) at this locus. Self-incompatibility is an obstacle for establishing pure lines and fixation of agronomically useful genes. Elucidation of the molecular mechanism of heterostylous self-incompatibility of common buckwheat has continued for a quarter of a century. Recent advances in genomic and transcriptomic analyses using next-generation sequencing have made it possible to determine the genomic region harboring the buckwheat *S* locus and to identify novel genes at this locus. In this review, we summarize the current knowledge on buckwheat heterostyly gained from conventional and molecular genetics and genomics. We also discuss the application of these studies to breeding of common buckwheat.

Key Words: common buckwheat, heteromorphic SI, self-incompatibility.

Introduction

Self-incompatibility (SI) prevents self-fertilization. It is widespread in the plant kingdom. SI promotes outbreeding and reduces the likelihood of adverse effects caused by homozygosity of recessive alleles at multiple loci (Charlesworth and Charlesworth 1987, Goldberg *et al.* 2010). SI can be classified as either homomorphic or heteromorphic. Plants with homomorphic SI have only a single type of flower morphology, whereas plants with heteromorphic SI have two (distyly) or three (tristyly) types. Distyly is common and found in at least 28 angiosperm families including Polygonaceae, Primulaceae, Passifloraceae and Linaceae, whereas tristyly is rare and found in a few angiosperm families such as Oxalidaceae (Barrett 2002, de Nettancourt 2001, Ganders 1979). SI is caused primarily by a reaction between haploid pollen grains or pollen tubes and diploid stigmas or styles, and can be classified as either gametophytic or sporophytic. In gametophytic SI, the SI phenotype of each pollen grain is determined by its own genotype. In sporophytic SI, the SI phenotype of the pollen is determined by the genotype of its diploid parent. Three homomorphic SI systems have been extensively studied at the molecular level (reviewed in Iwano and Takayama 2012): (1) gametophytic SI based on the SLF (SFB)/S-RNase system in Solanaceae, Rosaceae and Plantaginaceae, (2) gametophytic SI based on the PrpS/PrsS system in Papaveraceae, and (3) sporophytic SI based on the SP11 (SCR)/SRK system in Brassicaceae.

Recently, considerable progress in understanding of the molecular basis of heteromorphic SI has been made in heterostylous plant species including common buckwheat (*Fagopyrum esculentum*) (Li *et al.* 2015, 2016, Takeshima *et al.* 2019, Ushijima *et al.* 2012, Yasui *et al.* 2012, 2016). Genus *Fagopyrum* includes both SI and self-compatible (SC) species. Heteromorphic SI was broken down many times independently during diversification of *Fagopyrum* species (Nishimoto *et al.* 2003, Ohnishi and Matsuoka 1996, Ohsako and Ohnishi 2000, Yasui and Ohnishi 1998a, 1998b). Two wild *Fagopyrum* species are SC species with heteromorphic flowers (Ohsako and Ohnishi 1998). Thus, we expect that *Fagopyrum* species will be well suited for studying the molecular mechanism of heteromorphic SI and its evolutionary history.

SI is important for maintaining genetic diversity, but it is

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often an obstacle to establishing new pure lines. Common buckwheat needs pollinators such as bees and flies for cross-pollination between pin and thrum plants, and seed production is strongly influenced by pollinator activity. Self-fertilizing SC lines no longer need to attract pollinators. Thus, it is expected that the seed production of SC lines would be more stable than outcrossing varieties. It may also be possible to increase seed yield by reducing the cost of nectar production (Galetto *et al.* 2018).

Recently, we published a review for genetics of buckwheat heteromorphic SI (Ueno *et al.* 2016). In addition to the knowledge in the previous review, we compare genes controlling heteromorphic SI among distantly related species, buckwheat, *Primula*, and *Turnera* species. We also discuss the application of these studies to breeding of common buckwheat.

Heteromorphic SI and *S* supergene hypothesis in buckwheat

Buckwheat is a distylous SI plant. It has long-styled flowers with short stamens (so-called pin flowers, **Fig. 1A**) and short-styled flowers with long stamens (so-called thrum flowers, **Fig. 1B**) (Darwin 1877, Hilderbrand 1867). Pollen grains of thrum plants (i.e., plants setting thrum flowers) are larger than those of pin plants (Schoch-Bodmer 1934, Tatebe 1953). Intra-morph incompatibility occurs in the style: in crosses between thrum plants, thrum pollen tube growth is inhibited in the upper part of the style with hypertrophy at the tips of pollen tubes, whereas in crosses between pin plants, pin pollen tube growth is inhibited in the middle part of the style with no hypertrophy (Hirose *et al.* 1995) (**Fig. 2**). Both SI and flower morphology are controlled by a single locus (*S* locus); thrum plants are heterozygous (*Ss*), whereas pin plants are homozygous (*ss*), and the *SS* genotype does not exist (Garber and Quisenberry 1927, Lewis and Jones 1992).

Similar phenomenon has been studied in *Primula*, which has been known as a distylous plant since the Darwin's era (Darwin 1862). According to Dowrick (1956), the *S* locus in *Primula* contains five genes (*S* supergene complex locus) for the following traits, as suggested by the observed recombinations within the *S* supergene complex locus: the *style length* gene (*G* for short style, *g* for long style), *style incompatibility* gene (I^S for style incompatibility of short style, i^s for style incompatibility of long style), *pollen incompatibility* gene (I^P for pollen incompatibility of short anther, i^p for pollen incompatibility of long anther), *pollen size* gene (*P* for large pollen grain, *p* for small pollen grain) and *anther height gene* (*A* for long anther, and *a* for short anther). Based on this hypothesis, the *S* allele would consist of the GI^SI^PPA gene cluster (i.e., haplotype) and the *s* allele would consist of the gi^si^ppa haplotype, and these haplotypes would be inherited without recombination in most cases. Sharma and Boyes (1961) postulated that heteromorphic SI in common buckwheat is also controlled by the *S* supergene. However, unlike in *Primula*, recombinations within the supergene complex have not been observed in buckwheat, and the *S* supergene hypothesis remained unproven for a long time.

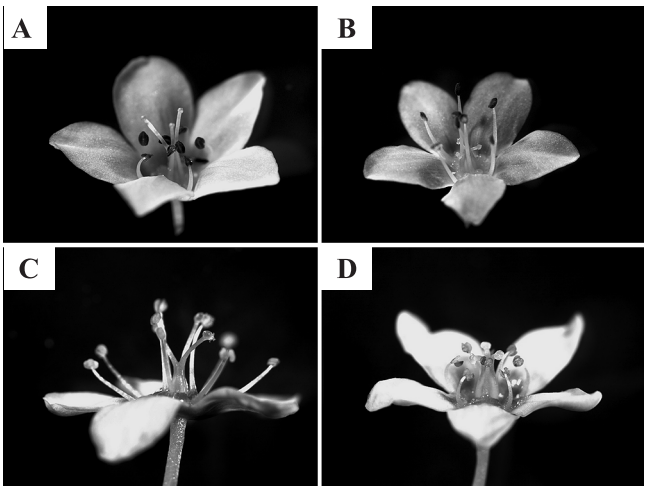

Fig. 1. Heteromorphic flowers of common buckwheat. (A) Pin flower; (B) Thrum flower; (C) Long-homostyle flower; (D) Short-homostyle flower.

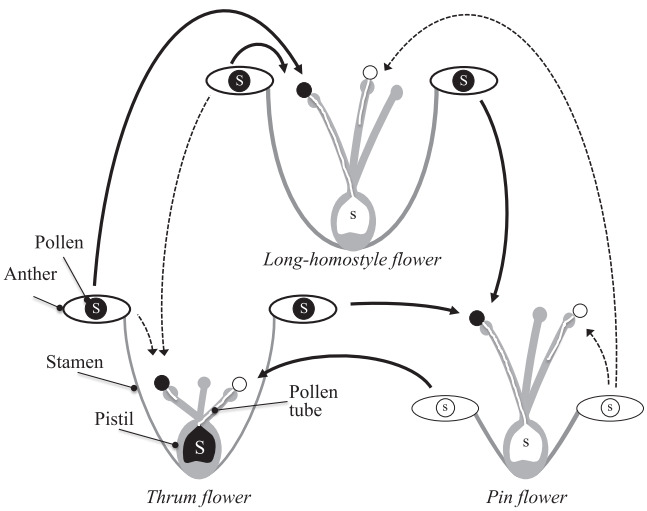

Fig. 2. Diagram of cross-compatibility/incompatibility among pin, thrum and long-homostyle plants. Buckwheat is sporophytic SI species, and the SI phenotype of the pollen is determined by the genotype of its diploid parent. Solid arrows, compatible crosses; dashed arrows, incompatible crosses.

Self-compatibility and S^h allele at the *S* locus of *Fagopyrum homotropicum*

The *S* supergene hypothesis was confirmed in common buckwheat by genetic studies that used crosses between common buckwheat and SC *Fagopyrum* species. An homostylous SC species closely related to common buckwheat, *Fagopyrum homotropicum* Ohnishi, was found in southern China (Ohnishi 1998), and several researchers have

succeeded in transferring the SC property of *F. homotropicum* to common buckwheat by conducting interspecific crosses between these two species (Aii *et al.* 1998, Campbell 1995, Matsui *et al.* 2003b, Woo *et al.* 1999). It has been proven that the SC property is conferred by the S^h allele at the *S* locus of *F. homotropicum*, and that S^h is dominant over the *s* allele but recessive to the *S* allele (Woo *et al.* 1999). Using interspecific crossing and subsequent selfing, Matsui *et al.* (2003b, 2007) established SC lines that had flowers with long styles, long stamens and large pollen ("long-homostyle flowers"; **Fig. 1C**). The SC lines had both the male phenotypes of thrum plants and the female phenotypes of pin plants: (1) pollen from SC lines was compatible with long styles of pin plants, but incompatible with short styles of thrum plants, and (2) long styles of SC lines were compatible with pollen from long stamens of thrum plants, but incompatible with pollen from short stamens of pin plants (**Fig. 2**). On the basis of these observations, Matsui *et al.* (2003b, 2007) proposed the presence of at least two factors in the *S* locus, one controlling style incompatibility and style length, and the other one controlling pollen incompatibility, pollen size and stamen height. They also postulated the genotype in the S^h haplotype to be gi^sI^PPA and that this genotype was caused by recombination in the *S* supergene, as proposed for *Primula* (Wedderburn and Richards 1992).

S-LOCUS EARLY FLOWERING 3 (*S-ELF3*) gene in the buckwheat *S* allele

Factors related to homomorphic SI systems in Brassicaceae, Solanaceae and Papaveraceae have been identified by searching for specific proteins in different *S* haplotypes, and the finding of proteins segregating with *S* haplotypes has led to the isolation of *S* genes (reviewed by Takayama and Isogai 2005).

In distylous plants including buckwheat, the dominance of the *S* allele over the *s* allele suggests that a specific gene is expressed only in the flowers of thrum plants. Yasui *et al.* (2012) isolated mRNAs separately from pistils of thrum and pin plants, and conducted high-throughput sequencing analyses of expressed genes. Four specific transcripts (SSG1–SSG4) were detected in the style of thrum plants. Using more than 1,000 BC_1F_5 segregating plants and an ion-beam-induced mutant, Yasui *et al.* (2012) found complete genetic linkage between the *S* locus and *SSG2* or *SSG3*. *SSG3* was found to be a homolog of *Arabidopsis EARLY FLOWERING 3* (*ELF3*) and was named *S-LOCUS EARLY FLOWERING 3* (*S-ELF3*). No homologs were identified for *SSG2* (Yasui *et al.* 2012).

ELF3, a core circadian clock component, forms a complex with ELF4 and LUX ARRHYTHMO (LUX); this complex binds to the promoters of circadian clock genes such as *PHYTOCHROME-INTERACTING FACTOR 4* (*PIF4*), *PIF5* and *PSEUDO-RESPONSE REGULATOR 9* and regulates their expression in a circadian manner (Helfer *et al.* 2011, Herrero *et al.* 2012). Because ELF3 functions in flowering repression (Liu *et al.* 2001, Zagotta *et al.* 1996), diurnal control of hypocotyl growth (Nusinow *et al.* 2011) and thermo-responsive hypocotyl growth (Box *et al.* 2015, Raschke *et al.* 2015), S-ELF3 function could be related to the regulation of style length.

Analysis of the *S* locus based on genome data and comparison of buckwheat and *Primula S* loci

Yasui *et al.* (2012) reported that the genomic region harboring *S-ELF3* is absent in the *s* haplotype in a worldwide collection of common buckwheat landraces and in two distantly related *Fagopyrum* species that have heteromorphic SI. Yasui *et al.* (2016) assembled the common buckwheat genome and suggested that the region harboring *S-ELF3* is present only in the *S* haplotype, that the *S* locus in buckwheat is also hemizygous, and that homostyly does not arise by recombination between the *S* and *s* alleles but is caused by dysfunction of the G/I^S or A/I^P gene of the *S* allele caused by mutations. Yasui *et al.* (2012) amplified *S-ELF3* from Kyushu PL4 (an SC line in which the S^h allele of SC *F. homotropicum* was incorporated into *F. esculentum*, Matsui *et al.* 2008) and showed that a single-nucleotide deletion in this line results in a frameshift in the 5th exon of *S-ELF3*. Dysfunction of S-ELF3, which is expressed in pistils and could be the G/I^S factor, would confer the phenotype of the *s* haplotype in pistils. Thus, we postulate that Kyushu PL4 possesses the dysfunctional G/I^S and functional A/I^P factors, giving it both the female phenotypes of pin plants and the male phenotypes of thrum plants (**Fig. 3**).

De novo assembly of the *Primula vulgaris* genome sequence and comparison of the genomes of pin and thrum plants have revealed that the *S* supergene is present only in

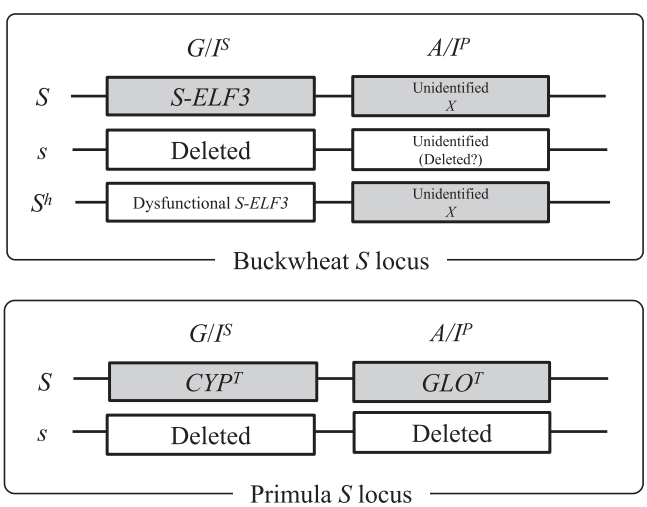

Fig. 3. Genes of the buckwheat and *Primula S* supergene. In thrum plants, the genomic region harboring the *S* supergene is hemizygous. *S-ELF3* is a candidate for G/I^S. The gene for A/I^P has not yet been identified.

the genome of thrum plants, indicating that thrum plants are hemizygous, not heterozygous, for the *S* locus (Li *et al.* 2015, 2016). The nucleotide length of hemizygous genomic region of the *S* locus is estimated to be 278 Kb (Li *et al.* 2016). They also suggested that two tightly linked genes in the *S* haplotype separately control anther and style length, and showed that a transposon insertion into GLO^T is associated with loss of anther elevation and that a CYP^T allele with a single-base insertion in exon 3 is associated with loss of style length suppression (i.e., exhibiting long style length) (**Fig. 3**). Yasui *et al.* (2016) also showed that the length of the *S*-allele region harboring *S-ELF3* is over 5.8 Mb. The genomic region controlling the heteromorphic SI system in buckwheat is longer than that in *P. vulgaris*. Thus, unlike the short *S*-allele region of *P. vulgaris*, the genomic region controlling the heteromorphic SI system is huge in buckwheat. Comparison between the *S*-allele regions of *Primula* and *Fagopyrum* will shed light on the convergent evolution of heteromorphic SI in these distantly related species.

Polygalacturonase as a gene related to heteromorphic SI

Using isoelectric focusing of nondenatured proteins, Athanasiou and Shore (1997) analyzed proteins differentially expressed between pin and thrum plants of *Turnera subulata*, and detected thrum-specific proteins of the style and pollen. Following two-dimensional (2D)-PAGE analysis, Athanasiou *et al.* (2003) determined the amino acid sequences of thrum-specific proteins. One protein was a member of the polygalacturonase (PG) family of pectin hydrolytic enzymes involved in the development of pollen and anther, floral morphology (Huang *et al.* 2009a, 2009b, Zhang and Ren 2008), cell elongation (Babu *et al.* 2013), seed germination (Sitrit *et al.* 1996, 1999), pod and anther dehiscence (Ogawa *et al.* 2009, Xiao *et al.* 2014) and fruit softening (Kramer and Redenbaugh 1994).

Matsui *et al.* (2004) surveyed SI responses and flower morphology of Pennline 10, a short-homostyle (**Fig. 1D**) line established by Marshall (1970), and found that the short pistils of this line have lost the SI response and are compatible with pollen from pin and thrum plants. Matsui *et al.* (2004) also confirmed that the lack of SI response and the short style length of Pennline 10 are controlled by multiple genes outside the *S* locus. Recently, Takeshima *et al.* (2019) compared protein profiles of upper pistils between pin and thrum plants by 2D-PAGE analysis, and detected short style-specific proteins. Amino acid sequence analysis showed that one of these proteins was a truncated form of PG, but the gene encoding this protein, *FePG1*, is not linked to the *S* locus. On the basis of *FePG1* expression in the styles of short-homostyle plants, Takeshima *et al.* (2019) suggested that *FePG1* is not an *S*-locus gene but acts downstream of the *S* locus to control style length. The genus *Turnera* (Turneraceae) is distantly related to *Fagopyrum*. Parallel recruitment of PGs in two divergent plant genera, *Fagopyrum* and *Turnera*, indicates the important role of PGs in the thrum phenotype in heteromorphic SI. Comparison between downstream networks including PGs will offer a chance to understand the molecular mechanism of convergent evolution of heteromorphic SI in these diversified plants.

Heteromorphic SI in *Fagopyrum* species

The genus *Fagopyrum* is composed of two major phylogenetic groups, Cymosum and Urophyllum, and SC species are present in both (**Table 1**). It is suggested that breakdown of heteromorphic SI occurred many times during *Fagopyrum* evolution (Nishimoto *et al.* 2003, Ohnishi and Matsuoka 1996, Ohsako and Ohnishi 2000, Yasui and Ohnishi 1998a, 1998b). Yasui *et al.* (2012) found that two SC plants, SC line of *F. esculentum* (Kyushu PL4) and *F. tataricum*, have nonsense mutations in *S-ELF3*, and suggested the importance of *S-ELF3* in the heteromorphic SI system in *Fagopyrum*. Determination of the gene structure of *S-ELF3* in other SC species will be crucial for elucidating its function in the heteromorphic SI system. Two heteromorphic SC species, *F. pleioramosum* and *F. callianthum*, are selfing wild plants sustaining heteromorphic flower morphs (Ohsako and Ohnishi 1998). It will be important to clarify whether they are prototypic heteromorphic SI species, or whether heteromorphic SI in these species has just recently been broken down by recombination within *S* alleles or mutations in genes that act downstream of those controlling SI.

In the Urophyllum group, some SI species, such as *F. statice* and *F. leptopodum*, have relatively small genomes (**Table 1**) (Nagano *et al.* 2000). The *S*-allele region in common buckwheat consists of 332 small scaffolds, mainly due to the genome complexity caused by the heteromorphic nature of buckwheat and the large genome (1.21 G) (Yasui

Table 1. Mating system and genome size of *Fagopyrum* species[a]

Phylogenetic group	Species	Mating system[b]	Genome size (Mbp)[c]
Cymosum group	*F. esculentum*	Heteromorphic SI[d]	1,340
	F. homotropicum	Homomorphic SC[d]	1,080
	F. cymosum	Heteromorphic SI	1,120
	F. tataricum	Homomorphic SC	540
Urophyllum group	*F. urophyllum*	Heteromorphic SI	1,850
	F. leptopodum	Heteromorphic SI	690
	F. statice	Heteromorphic SI	650
	F. gracilipes	Homomorphic SC	810
	F. pleioramosum	Heteromorphic SC	1,470
	F. capillatum	Heteromorphic SC	830

[a] Only species whose genome size was estimated in Nagano *et al.* (2000) are listed.
[b] As in Nishimoto *et al.* (2003).
[c] As in Nagano *et al.* (2000).
[d] SC, self-compatibility; SI, self-incompatibility.

et al. 2016). DenovoMAGIC 3.0 (Avni *et al.* 2017) and 10X Chromium technology (Zheng *et al.* 2016) can be adopted for assembling heteromorphic genome sequences. Species with small genomes are most suitable for obtaining sequences of the *S*-allele region. Because these species are found only in China, collaboration with Chinese groups would be important for comprehensive understanding of the structure of the *S*-allele region in *Fagopyrum*.

Potential application of the knowledge of the *S*-locus to buckwheat breeding

Buckwheat needs pollination between pin and thrum plants by bees or other insects to produce seeds. Pollination, and therefore yield, is strongly affected by environmental conditions. SI plants maintain high heterogeneity, and fixing agricultural traits is time consuming. Attempts to introduce SC traits from SC species into common buckwheat have been successful with *F. homotropicum* (Aii *et al.* 1998, Campbell 1995, Matsui *et al.* 2003b, Woo *et al.* 1999). However, plants produced by interspecific crosses have undesirable traits such as shattering and lodging. Matsui *et al.* (2003a) investigated the inheritance of the shattering habit and removed it by backcrossing with a leading SI common buckwheat cultivar, but more such crosses are needed to produce new cultivars desirable for farmers and consumers.

The S^h allele, conferring the SC trait, is dominant over the *s* allele but recessive to the *S* allele. Thus, to obtain F_1 plants with the SC trait, pin plants and SC lines need to be crossed. It is easy to determine whether a plant is SC or SI in F_2 and later generations from flower morphology, but two genotypes, S^h/S^h homozygous and S^h/s heterozygous, cannot be distinguished by flower morphology. Heterozygous plants would segregate SC and SI plants in the next generation. To accelerate buckwheat breeding, molecular markers to distinguish homo- and heterozygosity at the *S* locus in seedlings would be beneficial. Aii *et al.* (1998) developed AFLP-based STS markers linked to the S^h allele, yet the linkage distance was more than 5 cM. Because the *S* locus including *S-ELF3* is present only in thrum plants, it is impossible to develop co-dominant markers for *S-ELF3*. Developing co-dominant markers 'tightly linked' to the *S* locus would be possible, and the availability of such markers would accelerate breeding programs of buckwheat.

Recently we developed the Buckwheat Genome DataBase (BGDB, http://buckwheat.kazusa.or.jp). This database can be used for the rapid detection of homologues of genes previously identified in other plants, and we've detected agronomically useful genes encoding 2S albumin-type allergens and granule-bound starch synthases (Yasui *et al.* 2016). Now we are screening dysfunctional alleles of these genes from ethyl methanesulfonate (EMS)-induced mutant pools of buckwheat. In near future, we can establish SC lines with highly valuable agricultural traits, such as low-allergen or low amylose buckwheat lines.

Author Contribution Statement

KM wrote the main text and generated figures; YY wrote the main text and generated figures and tables.

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Literature Cited

Aii, J., M. Nagano, G. Penner, G.C. Campbell and T. Adachi (1998) Identification of RAPD markers linked to the *Homostylar* (*Ho*) gene in buckwheat. Breed. Sci. 48: 59–62.

Athanasiou, A. and J.S. Shore (1997) Morph-specific proteins in pollen and styles of distylous *Turnera* (Turneraceae). Genetics 146: 669–679.

Athanasiou, A., D. Khosravi, F. Tamari and J.S. Shore (2003) Characterization and localization of short-specific polygalacturonase in distylous *Turnera subulata* (Turneraceae). Am. J. Bot. 90: 675–682.

Avni, R., M. Nave, O. Barad, K. Baruch, S.O. Twardziok, H. Gundlach, I. Hale, M. Mascher, M. Spannagl, K. Wiebe *et al.* (2017) Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. Science 357: 93–97.

Babu, Y., T. Musielak, A. Henschen and M. Bayer (2013) Suspensor length determines developmental progression of the embryo in Arabidopsis. Plant Physiol. 162: 1448–1458.

Barrett, S.C. (2002) The evolution of plant sexual diversity. Nat. Rev. Genet. 3: 274–284.

Box, M.S., B.E. Huang, M. Domijan, K.E. Jaeger, A.K. Khattak, S.J. Yoo, E.L. Sedivy, D.M. Jones, T.J. Hearn, A.A.R. Webb *et al.* (2015) *ELF3* controls thermoresponsive growth in *Arabidopsis*. Curr. Biol. 25: 194–199.

Campbell, C. (1995) Inter-specific hybridization in the genus *Fagopyrum*. *In*: Matano, T. and A. Ujihara (eds.) Proceedings of the 6th International Symposium on Buckwheat, Shinshu University Press, Ina, pp. 255–263.

Charlesworth, D. and B. Charlesworth (1987) The effect of investment in attractive structures on allocation to male and female functions in plants. Evolution 41: 948–968.

Darwin, C. (1862) On the two forms or dimorphic condition in the species of *Primula*, and on their remarkable sexual relations. Bot. J. Linn. Soc. 6: 77–96.

Darwin, C. (1877) The different forms of flowers on plants of the same species. Murray, London.

de Nettancourt, D. (2001) Incompatibility and incongruity in wild and cultivated plants. Springer, Berlin.

Dowrick, V.P.J. (1956) Heterostyly and homostyly in *Primula obconica*. Heredity 10: 219–236.

Galetto, L., F.P. Araujo, G. Grilli, L.D. Amarilla, C. Torres and M. Sazima (2018) Flower trade-offs derived from nectar investment in female reproduction of two *Nicotiana* species (Solanaceae). Acta Bot. Brasilica 32: 473–478.

Ganders, F.R. (1979) The biology of heterostyly. N.Z. J. Bot. 17: 607–635.

Garber, R.J. and K.S. Quisenberry (1927) The inheritance of length of

style in buckwheat. J. Agric. Res. 34: 181–183.

Goldberg, E.E., J.R. Kohn, R. Lande, K.A. Robertson, S.A. Smith and B. Igic (2010) Species selection maintains self-incompatibility. Science 330: 493–495.

Helfer, A., D.A. Nusinow, B.Y. Chow, A.R. Gehrke, M.L. Bulyk and S.A. Kay (2011) *LUX ARRHYTHMO* encodes a night time repressor of circadian gene expression in the Arabidopsis core clock. Curr. Biol. 21: 126–133.

Herrero, E., E. Kolmos, N. Bujdoso, Y. Yuan, M.M. Wang, M.C. Berns, H. Uhlworm, G. Coupland, R. Saini, M. Jaskolski *et al.* (2012) EARLY FLOWERING4 recruitment of EARLY FLOWERING3 in the nucleus sustains the *Arabidopsis* circadian clock. Plant Cell 24: 428–443.

Hilderbrand, F. (1867) Die Geschlechter-vertheilung bei den pelanzen und das gesetz der vermiedene und unvortheilhaften stetigen selbstbefruchtung. Verlag von Wilhelm Engelmann, Leipzig.

Hirose, T., A. Ujihara, H. Kitabayashi and M. Minami (1995) Pollen tube behavior related to self-incompatibility in interspecific crosses of *Fagopyrum*. Breed. Sci. 45: 65–70.

Huang, L., J.S. Cao, A.H. Zhang, Y.Q. Ye, Y.C. Zhang and T.T. Liu (2009a) The polygalacturonase gene *BcMF2* from *Brassica campestris* is associated with intine development. J. Exp. Bot. 60: 301–313.

Huang, L., Y.Q. Ye, Y.C. Zhang, A.H. Zhang, T.T.T. Liu and J.S. Cao (2009b) *BcMF9*, a novel polygalacturonase gene, is required for both *Brassica campestris* intine and exine formation. Ann. Bot. 104: 1339–1351.

Iwano, M. and S. Takayama (2012) Self/non-self discrimination in angiosperm self-incompatibility. Curr. Opin. Plant Biol. 15: 78–83.

Kramer, M.G. and K. Redenbaugh (1994) Commercialization of a tomato with an antisense polygalacturonase gene: The FLAVR SAVR™ tomato story. Euphytica 79: 293–297.

Lewis, D. and D.A. Jones (1992) The genetics of heterostyly. *In*: Barrett, S.C.H. (ed.) Evolution and Function of Heterostyly. Springer-Verlag, Berlin Heidelberg, pp. 129–150.

Li, J.H., M.A. Webster, J. Wright, J.M. Cocker, M.C. Smith, F. Badakshi, P. Heslop-Harrison and P.M. Gilmartin (2015) Integration of genetic and physical maps of the *Primula vulgaris S* locus and localization by chromosome *in situ* hybridization. New Phytol. 208: 137–148.

Li, J.H., J.M. Cocker, J. Wright, M.A. Webster, M. McMullan, S. Dyer, D. Swarbreck, M. Caccamo, C. van Oosterhout and P.M. Gilmartin (2016) Genetic architecture and evolution of the *S* locus supergene in *Primula vulgaris*. Nat. Plants 2: 16188.

Liu, X.L., M.F. Covington, C. Fankhauser, J. Chory and D.R. Wanger (2001) *ELF3* encodes a circadian clock-regulated nuclear protein that functions in an Arabidopsis *PHYB* signal transduction pathway. Plant Cell 13: 1293–1304.

Marshall, H.G. (1970) Registration of 'Pennline 10' buckwheat. Crop Sci. 10: 726.

Matsui, K., T. Tetsuka and T. Hara (2003a) Two independent gene loci controlling non-brittle pedicels in buckwheat. Euphytica 134: 203–208.

Matsui, K., T. Tetsuka, T. Nishio and T. Hara (2003b) Heteromorphic incompatibility retained in self-compatible plants produced by a cross between common and wild buckwheat. New Phytol. 159: 701–708.

Matsui, K., T. Nishio and T. Tetsuka (2004) Genes outside the *S* supergene suppress S functions in buckwheat (*Fagopyrum esculentum*). Ann. Bot. 94: 805–809.

Matsui, K., T. Nishio and T. Tetsuka (2007) Use of self-compatibility and modifier genes for breeding and genetic analysis in common buckwheat (*Fagopyrum esculentum*). Jpn. Agric. Res. Q. 41: 1–5.

Matsui, K., T. Tetsuka, T. Hara and T. Morishita (2008) Breeding and characterization of a new self-compatible common buckwheat parental line, "Buckwheat Norin-PL1". Bull. Natl. Agric. Res. Cent. Kyushu Okinawa Reg. 49: 1–17.

Nagano, M., J. Aii, C.G. Campbell, S. Kawasaki and T. Adachi (2000) Genome size analysis of the genus *Fagopyrum*. Fagopyrum 17: 35–39.

Nishimoto, Y., O. Ohnishi and M. Hasegawa (2003) Topological incongruence between nuclear and chloroplast DNA trees suggesting hybridization in the urophyllum group of the genus *Fagopyrum* (Polygonaceae). Genes Genet. Syst. 78: 139–153.

Nusinow, D.A., A. Helfer, E.E. Hamilton, J.J. King, T. Imaizumi, T.F. Schultz, E.M. Farre and S.A. Kay (2011) The ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth. Nature 475: 398–402.

Ogawa, M., P. Kay, S. Wilson and S.M. Swain (2009) ARABIDOPSIS DEHISCENCE ZONE POLYGALACTURONASE1 (ADPG1), ADPG2, and QUARTET2 are polygalacturonases required for cell separation during reproductive development in *Arabidopsis*. Plant Cell 21: 216–233.

Ohnishi, O. and Y. Matsuoka (1996) Search for the wild ancestor of buckwheat II. Taxonomy of *Fagopyrum* (Polygonaceae) species based on morphology, isozymes and cpDNA variability. Genes Genet. Syst. 71: 383–390.

Ohnishi, O. (1998) Search for the wild ancestor of buckwheat III. The wild ancestor of cultivated common buckwheat, and of tatary buckwheat. Econ. Bot. 52: 123–133.

Ohsako, T. and O. Ohnishi (1998) New *Fagopyrum* species revealed by morphological and molecular analyses. Genes Genet. Syst. 73: 85–94.

Ohsako, T. and O. Ohnishi (2000) Intra- and interspecific phylogeny of wild *Fagopyrum* (Polygonaceae) species based on nucleotide sequences of noncoding regions in chloroplast DNA. Am. J. Bot. 87: 573–582.

Raschke, A., C. Ibanez, K.K. Ullrich, M.U. Anwer, S. Becker, A. Glockner, J. Trenner, K. Denk, B. Saal, X.D. Sun *et al.* (2015) Natural variants of *ELF3* affect thermomorphogenesis by transcriptionally modulating *PIF4*-dependent auxin response genes. BMC Plant Biol. 15: 197.

Schoch-Bodmer, H. (1934) Zum Heterostylieproblem: Griffelbeschaffenheit und Pollenschlauchwachstum bei *Fagopyrum esculentum*. Planta 22: 149–152.

Sharma, K.D. and J.W. Boyes (1961) Modified incompatibility of buckwheat following irradiation. Can. J. Bot. 39: 1241–1246.

Sitrit, Y., B. Downie, A.B. Bennett and K.J. Bradford (1996) A novel exo-polygalacturonase is associated with radicle protrusion in tomato (*Lycopersicon esculentum*) seeds. Plant Physiol. 111: 752–752.

Sitrit, Y., K.A. Hadfield, A.B. Bennett, K.J. Bradford and A.B. Downie (1999) Expression of a polygalacturonase associated with tomato seed germination. Plant Physiol. 121: 419–428.

Takayama, S. and A. Isogai (2005) Self-incompatibility in plants. Annu. Rev. Plant Biol. 56: 467–489.

Takeshima, R., T. Nishio, S. Komatsu, N. Kurauchi and K. Matsui (2019) Identification of a gene encoding polygalacturonase expressed specifically in short styles in distylous common buckwheat (*Fagopyrum esculentum*). Heredity (Edinb) 123: 492–502.

Tatebe, T. (1953) Physiological research on the fertility of the buckwheat. III. On the self-fertile, long-styled plants. Japan. J. Breed.

2: 240–244.

Ueno, M., Y. Yasui, J. Aii, K. Matsui, S. Sato and T. Ota (2016) Genetic analyses of the heteromorphic self-incompatibility (*S*) locus in buckwheat. *In*: Zhou, M. (ed.) Molecular breeding and nutritional aspects of buckwheat. Academic Press, pp. 411–422.

Ushijima, K., R. Nakano, M. Bando, Y. Shigezane, K. Ikeda, Y. Namba, S. Kume, T. Kitabata, H. Mori and Y. Kubo (2012) Isolation of the floral morph-related genes in heterostylous flax (*Linum grandiflorum*): the genetic polymorphism and the transcriptional and post-transcriptional regulations of the *S* locus. Plant J. 69: 317–331.

Wedderburn, F.M. and A.J. Richards (1992) Secondary homostyly in *Primula* L.; evidence for the model of the *S* supergene. New Phytol. 121: 649–655.

Woo, S.H., T. Adachi, S.K. Jong and C.G. Campbell (1999) Inheritance of self-compatibility and flower morphology in an interspecific buckwheat hybrid. Can. J. Plant Sci. 79: 483–490.

Xiao, C., C. Somerville and C.T. Anderson (2014) POLYGALACTURONASE INVOLVED IN EXPANSION1 functions in cell elongation and flower development in *Arabidopsis*. Plant Cell 26: 1018–1035.

Yasui, Y. and O. Ohnishi (1998a) Interspecific relationships in *Fagopyrum* (Polygonaceae) revealed by the nucleotide sequences of the *rbcL* and *accD* genes and their intergenic region. Am. J. Bot. 85: 1134–1142.

Yasui, Y. and O. Ohnishi (1998b) Phylogenetic relationships among *Fagopyrum* species revealed by nucleotide sequences of the ITS region of the nuclear rRNA gene. Genes Genet. Syst. 73: 201–210.

Yasui, Y., M. Mori, J. Aii, T. Abe, D. Matsumoto, S. Sato, Y. Hayashi, O. Ohnishi and T. Ota (2012) *S-LOCUS EARLY FLOWERING 3* is exclusively present in the genomes of short-styled buckwheat plants that exhibit heteromorphic self-incompatibility. PLoS ONE 7: e31264.

Yasui, Y., H. Hirakawa, M. Ueno, K. Matsui, T. Katsube-Tanaka, S.J. Yang, J. Aii, S. Sato and M. Mori (2016) Assembly of the draft genome of buckwheat and its applications in identifying agronomically useful genes. DNA Res. 23: 215–224.

Zagotta, M.T., K.A. Hicks, C.I. Jacobs, J.C. Young, R.P. Hangarter and D.R. Meeks-Wagner (1996) The *Arabidopsis ELF3* gene regulates vegetative photomorphogenesis and the photoperiodic induction of flowering. Plant J. 10: 691–702.

Zhang, X.H. and Y. Ren (2008) Floral morphology and development in *Sargentodoxa* (Lardizabalaceae). Int. J. Plant Sci. 169: 1148–1158.

Zheng, G.X., B.T. Lau, M. Schnall-Levin, M. Jarosz, J.M. Bell, C.M. Hindson, S. Kyriazopoulou-Panagiotopoulou, D.A. Masquelier, L. Merrill, J.M. Terry *et al.* (2016) Haplotyping germline and cancer genomes with high-throughput linked-read sequencing. Nat. Biotechnol. 34: 303–311.