The European Journal of Plant Science and Biotechnology ***©2009 Global Science Books***

Floral Biology of Common Buckwheat (*Fagopyrum esculentum* Moench)

Valérie Cawoy[1] • Jean-François Ledent[2] • Jean-Marie Kinet[3] • Anne-Laure Jacquemart[1*]**

[1] Research group « Genetics, populations, reproduction » - Unité de Génétique, Université Catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium
[2] Unité d'écophysiologie et amélioration végétale, Université Catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium
[3] Unité de Biologie Végétale, Université Catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium

***Corresponding authors*: * anne-laure.jacquemart@uclouvain.be ** valerie.cawoy@uclouvain.be**

ABSTRACT

Common buckwheat (*Fagopyrum esculentum* Moench) is widely cultivated but the floral biology of this auto-incompatible distylous species was poorly documented. This review, based on our recent results obtained with the cultivar 'La Harpe' and the literature, summarizes main aspects related to its reproduction: morphogenesis, flowering, nectar, pollination and flower functionality. The activity of the reproductive meristems is potentially endless but the morphogenesis is stopped by the abortion processes. When the plant has one unfolded true leaf, the morphogenesis of the reproductive structures occurs at many nodes and this developmental stage is highly sensitive to environmental stress. *Hymenoptera* (*Apis mellifera* L. and *Bombus* spp.) and *Diptera* (*Syrphidae*) are the main visitors. Honeybee appears to be an effective pollinator and the most numerous visitors in Western Europe. At the anthesis, 'hexoses-dominant' floral nectar is secreted by unicellular secretory hairs during all the light period even after pollination. Except for the morphology of the reproductive organs, both morphs differ only in the nectar production. Thrum flowers secrete more nectar than pin flowers. Pollinator abundance is an important parameter to secure the yield but even without pollen transfer limitation the seed set is low. Flower fate appears to be dependent on a mechanism internal to the raceme which controls the percentage of flowers able to set a seed. Infertile flowers with an undersized gynoecium and last abortions after fecundation are not frequent. In contrast, flowers with a normal sized gynoecium which do not set seed after compatible pollination are the main contribution to the low seed set. This female organ sterility seems to be the most yield limiting factor throughout the world.

Keywords: distyly, morphogenesis, nectar, pollination, resource, seed set

CONTENTS

INTRODUCTION

Common buckwheat, *Fagopyrum esculentum* Moench, is one of the oldest domesticated crops of Asia. This crop was very popular food during 17th-19th century and was later abandoned during the 20th century in Western countries because of the competition with wheat. Common buckwheat has a promising future on a worldwide scale due to recent discoveries on its nutritional qualities. It is a multi-food-use pseudocereal with a better nutritional value than many common cereals and a functional food with industrial applications (Hagels 1999; Tomotake *et al*. 2000; Bonafaccia *et al.* 2003a, 2003b; Kawa *et al.* 2003; Kim *et al.* 2004; Krkošková and Mrázová 2005; Christa and Soral-Śmietana 2008; Liu *et al.* 2008; Tang *et al.* 2009). Therefore, buckwheat which is mainly consumed in Asia and in Eastern Europe is an alternative to common cereals in Western countries and receives increasing attention. This situation motivates new breeding programs and further researches to increase productivity of buckwheat achenes.

Up to now, seeds are the most important product and the success of the harvest depends on the flower number, the seed weight and the seed set. The evolution from the wild ancestor, *F. esculentum* ssp. *ancestrale*, towards present-day cultivars drifted from *F. esculentum* ssp. *esculentum*, has considerably modified plant morphology (Lakhanov and Napolova 2001). The wild species has a stronger main stem with many axillaries, a more developed root system and higher photosynthetic leaf area than modern cultivars (Lakhanov and Napalova 2001; Chen *et al.* 2004; Logacheva *et al.* 2008). Successive selections allowed the elimination of the strong seed dormancy and the abscission layer of the pedicel, the limitation of lodging, the increase of seed set and seed weight (Kreft 1989; Campbell 1997;

Received: 22 April, 2009. Accepted: 29 November, 2009.

Invited Review

Funatsuki *et al.* 2000; Lakhanov and Napolova 2001; Wagatsuma 2004). These numerous improvements turned out to be insufficient to obtain yields allowing buckwheat to compete with the other cereals. Actually, the mean yield in t ha^{-1} at world scale is for buckwheat 0.86, rye 2.21, wheat 2.80, rice 4.11 and maize 4.81 (FAOSTAT data, 2006).

Increasing productivity and yield stability, as well as improving the efficiency of breeding programmes, requires to understand the parameters that affect the duration of the flowering period, the pollination and the seed set. Concerning the reproductive mechanisms, buckwheat is a complex and exciting crop because of its numerous particularities. It is a hermaphroditic species with an indeterminate growth which produce self-incompatible distylous flowers pollinated by insects (Nagatomo and Adachi 1985). These characteristics make the improvement of buckwheat productivity complex, but their understanding will help to increase yields.

In this paper, we summarize the main aspects related to the reproduction of buckwheat: morphogenesis, flowering, nectar production, pollination and flower functionality. Most recent results reported here (Quinet *et al.* 2004; Halbrecq *et al.* 2005; Cawoy *et al.* 2006a, 2006b, 2007; Cawoy 2007; Jacquemart *et al.* 2007; Cawoy *et al.* 2008) were obtained with the cultivar 'La Harpe'. This diploid cultivar, adapted to the climate of temperate Western Europe, was developed and commercialized by INRA (Institut National de la Recherche Agronomique, France).

REPRODUCTIVE STRUCTURE INITIATION AND DEVELOPMENT

Growth of buckwheat is indeterminate and morphogenetic processes implicated in the establishment of the reproductive structures are potentially endless (Marshall and Pomeranz 1982; Quinet *et al.* 2004). They are dependent on the activity of meristems with an indeterminate mode of functioning which account for the anthesis potentialities of buckwheat to extend its flowering phase for protracted time period until abortion processes end the production of reproductive structures (i.e. inflorescence, cyme and flower; Quinet *et al.* 2004). These processes are regulated by resource availability. Actually, treatments which alter source/sink organ ratio during inflorescence morphogenesis by excision of selected inflorescences and/or leaves, respectively enhance or reduce the number of inflorescences and the number of flowers per inflorescence. Moreover seed development on a plant affect plant height, number of axillaries and reproductive morphogenesis in inflorescences, limiting the production of inflorescences and flowers (Racys and Montvilienne 2005; Cawoy *et al.* 2007).

Buckwheat initiates flowers over a wide range of daylengths (Nagatomo and Adachi 1985) and even under continuous light (Hao *et al.* 1995), but cultivars do not similarly react to photoperiod. Some cultivars, as the Japanese summer cultivars, seem non-sensitive to photoperiod whereas others, as the Japanese autumn cultivars and the European cultivar 'La Harpe', behave as facultative short-day plants (Minami and Namai 1986; Lachman and Adachi 1990; Hagiwara *et al.* 1998; Michiyama *et al.* 1998, 2003; Quinet *et al.* 2004). For photosensitive cultivars, flower initiation is advanced by short days compared to long days (i.e. it occurs at lower nodes) but more inflorescences and more flowers per inflorescence are initiated and the flowering period is longer under long days. The floral transition occurs when the first true leaf is expended (Quinet *et al.* 2004). At this developmental stage, buckwheat is very sensitive to any environmental stresses. For example, a low osmotic stress (4 days of hydroponic culture in a solution with polyethylene glycol, -0.078 Mpa), temperatures between –1 to –3°C for 4-6 h or a one day flooding are lethal or reduce the number of flowers by affecting the activity of the reproductive meristems involved in the production of cymes and flowers (Sugimoto and Sato 2000; Kalinová and Moudrý 2003; Cawoy *et al.* 2006b).

The shoot apical meristem produces two types of metamers (Quinet *et al.* 2004). Firstly, during the vegetative phase, metamers consist of a node with a leaf developing an ochrea and of an axillary meristem whose growth is delayed until floral transition occurs at higher nodes. Then, during the reproductive phase, the shoot apical meristem shifts to the production of metamers, each consisting of a node with a leaf or a bract at the higher nodes and a precocious axillary meristem which will develop into an inflorescence (**Fig. 1B, 1C**). After the production of 5-30 metamers, the arrest of functioning of the shoot apical meristem is accompanied by the abortion of the last inflorescence produced.

Buckwheat inflorescence is a compound raceme that produces laterally 1-30 uniparous cymes (**Fig. 1D**; Marshall and Pomeranz 1982; Nagatomo and Adachi 1985; Quinet *et al.* 2004). Inflorescences are situated at the extremity of the peduncles coming from the axil of the leaves or grouped in a terminal cluster at the extremity of the axes (main stem and axillaries; **Fig. 1A**). Peduncle sometimes bears two or rarely three inflorescences. The arrest of the morphogenetic activity of the raceme meristem and the abortion of the last formed cymes terminates the construction of the inflorescence (Quinet *et al.* 2004). Cymes develop in the axil of a bract wrapping 1-20 young flower buds that are initiated sequentially at the base of the pedicel of the preceding flower (**Fig. 1D**). Morphogenesis of the cymes stops with the abortion of some latest formed flowers.

Flowers are white to pink and the perianth is planar (perpendicular to the pedicel) at anthesis with a diameter about 6-7 mm (**Fig. 1E**; Campbell 1997; Cawoy *et al.* 2006a). It is formed of five petaloid tepals (not joined together; **Fig. 1G**). The androcium is formed of two whorls of stamens (**Fig. 1G**). Five stamens with introrse dehiscence form the external cycle and three stamens with extrorse dehiscence form the internal cycle (Campbell 1997). The stamens of the internal cycle are about 1.2 times longer than those of the external cycle and they contain about 7.5% more pollen grains (Namai and Fujita 1995). The gynoecium is formed of a superior unilocular ovary containing an orthotropous ovule and is surmounted by three styles (Campbell 1997). At the base of the ovary and alternating with the stamens, eight glands secrete nectar (**Fig. 1G**).

FLOWERING AND SEED RIPENING

Flowering starts 4 to 6 weeks after sowing and goes on during 4 to 15 weeks (Hedtke 1996; Naumkin 1998; Alekseyeva and Bureyko 2000; Halbecq *et al.* 2005; Cawoy *et al.* 2006a). Flowers are usually in anthesis only one day (Nagatomo and Adachi 1985; Quinet *et al.* 2004). The first anthesis occurs on the lowest inflorescence appearing on the 3rd to 5th node above the cotyledons of the main stem and the flowering progresses acropetally (from the base towards the top) along this axis (**Fig. 1A**; Quinet *et al.* 2004). Under this inflorescence, the flowering of axillaries progresses basipetally (from the top towards the base) along the main stem whereas along the axillaries flowering progresses acropetally (**Fig. 1A**). At the inflorescence scale, flowering starts with the anthesis of the first flower formed in the first cyme (Nagatomo and Adachi 1985; Quinet *et al.* 2004). Flowering continues from the base towards the top of the inflorescence (**Fig. 1E**), and within the cymes few days after anthesis of the first flower, another flower enters in anthesis (Nagatomo and Adachi 1985; Quinet et *al.* 2004). A peak of flowering which corresponds to anthesis of the maximum number of flowers per day occurs about 2 to 3 weeks after the first anthesis, and at this time plant growth stops (Naumkin 1998; Murakami *et al.* 2002; Halbrecq *et al.* 2005; Cawoy *et al.* 2006a).

After pollination, about 10 days are required before the embryo reaches its maximal size and two more weeks are needed for grain maturity (Obendorf *et al.* 1993). Guan and Adachi (1992) reported differences in growth rate of embryo according to seasons. The zygote divides more actively in summer than in autumn, and three days after pollination its

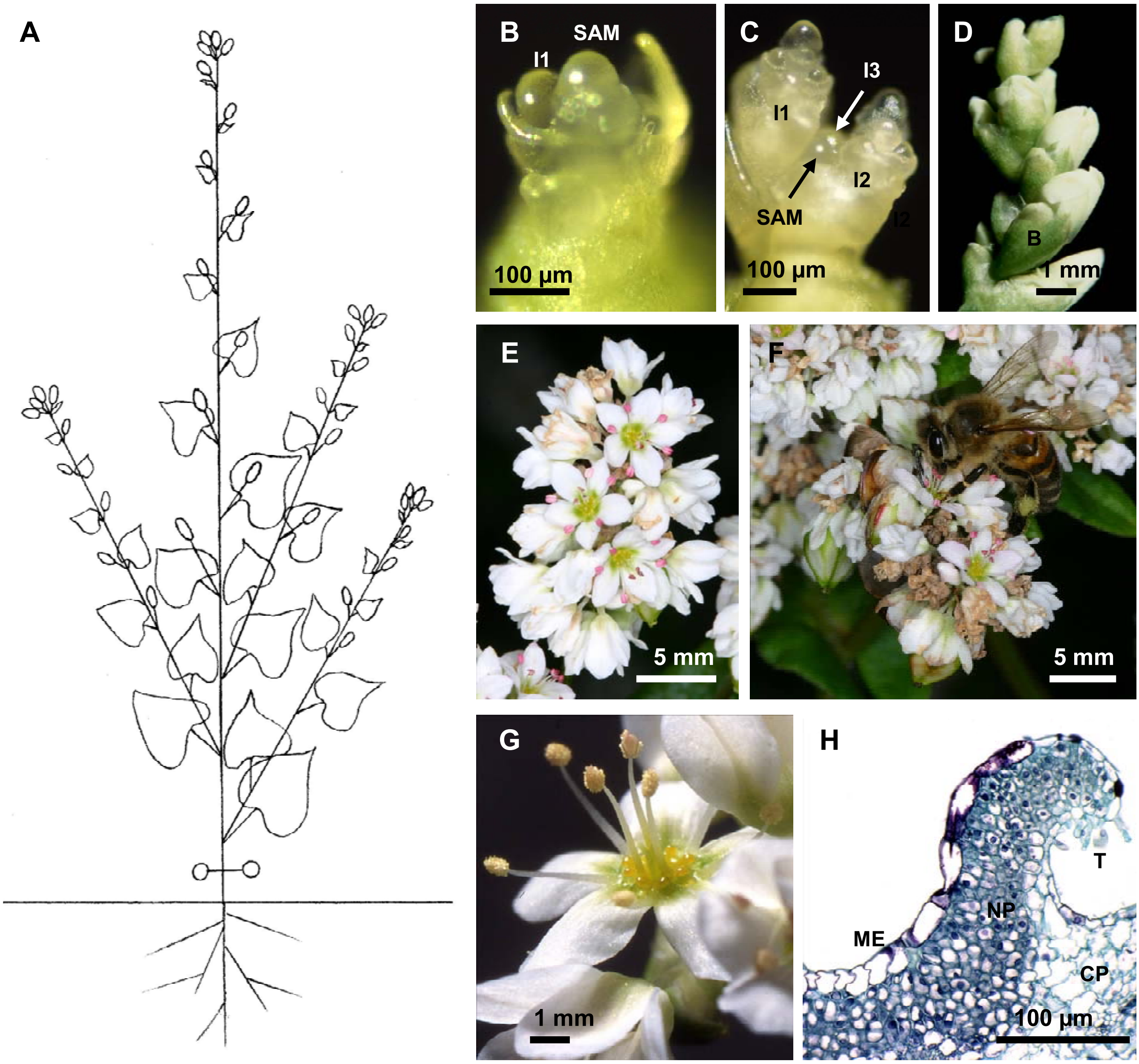

Fig. 1 Morphology of buckwheat reproductive structures. (**A**) Schematic drawing of plant architecture (round: cotyledon; oval: inflorescence). (**B**) Front macroscopic view of a shoot apex aged of 12 days. The shoot apical meristem (SAM) initiates a first inflorescence primordium (I1). (**C**) Front macroscopic view of a shoot apex aged of 17 days. The shoot apical meristem (SAM) and the first inflorescences (I1, I2 and I3) are visible. (**D**) Compound raceme before flowering make up of laterally uniparous cymes. In each cyme, a green bract (B) wraps flower buds. (**E**) Inflorescence when the flowering has reached the last cyme. (**F**) Honeybee which drink nectar on an inflorescence. Fruits at the different ripening stage (green to brown), flowers in anthesis and wilted flowers are visible. (**G**) Thrum flower in anthesis. The eight yellow nectaries secrete exposed nectar-forming drops on the receptacle. (**H**) Longitudinal section of a nectary. The hook-shaped nectary consists of a specialized multilayered parenchyma, the nectary parenchyma (NP), covered by a monolayered epidermis (ME) consisting of unicellular secretory trichomes (T) at the ventral face of the nectary. The sub-nectary parenchyma with some chloroplasts (CP) is also visible.

size is 1.7 times larger under autumn conditions. The fruit (the seed) is a triangular achene that measures about 5 mm long. Under the pericarp, the endosperm is covered by a fine testa and the embryo is situated at the centre of the endosperm (Marshall and Pomeranz 1982). Pericarp which is green during seed filling becomes brown then grey when the seed is mature (**Fig. 1F**). Due to the profusion of flowers (tens in anthesis per plant and per day), the long flowering period and consequently the long ripening period (seeds at all stages of ripening coexist within plants and inflorescences; **Fig. 1F**), choice of appropriate harvesting time is difficult (Marshall and Pomeranz 1982; Funatsuki *et al.* 2000). Moreover, there is no abscission layer but the pedicels are delicate and wind may cause scattering of grains on the soil (Campbell 1997; Funatsuki *et al.* 2000).

During flowering, temperature influences yields through impacts on development and fertility of the reproductive structures (Marshall and Pomeranz 1982; Adachi *et al.* 1983; Björkman 2000). Due to its frost sensitivity, buckwheat culture is restricted to low altitudes in temperate areas and harvest must be done before early frosts (Marshall and Pomeranz 1982; Björkman 2000). The optimal temperature for its growing is between 18 and 23°C. The flowering is inhibited below 15°C (delayed initiation and reduction of flower number) and flowers wither early at 10°C (Tahir and Farooq 1988; Lachman and Adachi 1990; Jessop *et al.* 1998; Gang and Yu 1998; Slawinska and Obendorf 2001). High temperatures (≥ 25°C) and dry winds cause flower withering, abortion of reproductive structures in development, malformations of embryo sac and fruit desiccation (Krotov 1963 *in* Campbell 1997; Nagatomo and Adachi 1985; Guan and Adachi 1992, 1994; Gang and Yu

1998; Slawinska and Obendorf 2001). During flowering and seed maturation, limiting water supply and flooding are damaging to endosperm development. They may cause embryo abortion and lighter mature seeds (Marshall and Pomeranz 1982; Lakhanov 1991; Kalinová *et al.* 2002; Sugimoto and Sato 2000). In order to stay viable, pollen needs humidity. Viability is lost in 1h when pollen is placed at 23°C in dry atmosphere (Adhikari and Campbell 1998). Slawinska and Obendorf (2001) mention a reduction of 45% of fertilization events when pollen comes from plants grown at 25°C instead of 18°C.

DISTYLY AND SELF-INCOMPATIBILITY

Buckwheat possesses a sexual dimorphism: each population is composed of plants with pin (long pistil and short stamens: styles that project 0.5-1 mm over the anthers) flowers and thrum (short pistil and long stamens: styles reach about the level of the middle of the filaments of the anthers) flowers (Nagatomo and Adachi 1985; Campbell 1997; Quinet *et al.* 2004). As in most distylous species (Lewis and Jones 1992), the action of the genes which control heterostyly in buckwheat is confined to the floral parts of the plants affecting mainly gynoecium and stamens. Actually, buckwheat morphology (proportion of different organ biomass: laminas, main stem and petioles, inflorescences and roots), number of reproductive structures and flowering behaviour are not affected by floral morph (Quinet *et al.* 2004; Cawoy *et al.* 2006a, 2008). While thrum flowers produce 0.7 times fewer pollen grains than pin flowers (pollen grains per stamen: thrum: 120-150, pin: 190-210; Ganders 1979; Namai and Fujita 1995; Cawoy *et al.* 2006a), and pollen grains of the thrum flowers are 1.4 times larger than those of the pin flowers (thrum: 50-55 µm; pin: 34-40 µm; Marshall and Pomeranz 1982; Samborska-Ciannia *et al.* 1989b; Namai and Fujita 1995; Cawoy *et al.* 2006a). The exine sculpturing and the size of the stigmatic cells differ also between the two morphs (Bahadur *et al.* 1984; Samborska-Ciannia *et al.* 1989b). Authors mention that the perianth size of buckwheat is generally larger in thrum than in pin flowers and report seed weight differences between morphs (Komenda *et al.* 1986; Lee 1986; Namai 1990b). However, Cawoy *et al.* (2006a) do not confirm these observations but find differences in nectar production (see below).

Loci controlling heteromorphism of the distylous species are closely related to loci controlling sporophytic incompatibility reactions. They are inherited together like one diallelic gene, called supergene *S*, which segregates as a simple Mendelian character (Ganders 1979). Sharma and Boyes (1961) postulated that supergene *S* is composed of 5 genes: *G* (style length); I^S (stylar incompatibility); I^P (pollen incompatibility); *P* (pollen size) and *A* (anther height, i.e. filament length). According to Matsui *et al.* (2003), I^S is tightly linked or pleiotropic with *G*, and I^P is tightly linked or pleiotropic with *A*. Common buckwheat is therefore strictly self-incompatible (Nagatomo and Adachi 1985; Adachi 1990). The genetic model represents thrum genotype as *Ss* (GI^SI^PA/gi^Si^Ppa) and pin genotype as *ss* (gi^Si^Ppa/gi^Si^Ppa). This implies that a 1:1 ratio between the two morphs is conserved from generation to generation. Studies on *Fagopyrum homotropicum*, a homostylous and self-compatible species discovered ten years ago, and on hybrids between *F. esculetum* and *F. homotropicum*, revealed that the genetic control of the floral morphology and compatibility is not limited to the super gene *S*. The allele controlling homomorphic flowers was designated as S^h (Woo *et al.* 1999). Authors suggest that it is derived from recombination in the supergene *S* (Matsui *et al.* 2003). Therefore, at a first locus is located the supergene *S* with three alleles and their intrallelic interaction $S > S^h > s$ (Woo *et al.* 1999). At a second locus would be located a diallelic gene (S_c) responsible for the morphology of the reproductive organs (Matsui *et al.* 2004; Wang *et al.* 2005). The two complementary dominant genes S^h and S^c control self-compatibility in *F. homotropicum*. To map S^h allele in buckwheat, RAPD (Random Amplified Length Polymorphism) and AFLP (Amplified Fragment Length Polymorphism) markers are presently available (Aii *et al.* 1998; Nagano *et al.* 2001; Yasui *et al.* 2004).

Following incompatible self-pollination (intra-morph pollination), pollen tube growth is inhibited at two-third of the style length in the long styles of pin flowers, and at the junction between the stigma and the style in the short styles of thrum flowers (Schoch-Bodmer 1934). In consequence, pollen tubes stop at nearly the same distance from the ovary in both morphs. Miljuš-Đukić *et al.* (2003, 2004) detected some proteins in the styles 2 h after compatible and incompatible pollinations. The proteins responsible for the self-incompatibility response are not the same in the long and the short styles. These authors assume that some of the proteins have probably a role in pollen adhesion to the stigma surface or/and in the inhibition of the pollen tube elongation.

NECTAR PRODUCTION

Efficiency of pollination depends on a plant's capacity to attract pollinators by flower morphology, and by pollen and nectar productions. In buckwheat, nectar production can be influenced by heteromorphy, ploidy level, plant age, inflorescence position and abiotic factors (Tahir and Farooq 1988; Namai 1990a, 1990b; Alekseyeva and Bureyko 2000; Cawoy *et al.* 2006a; Jacquemart *et al.* 2007; Cawoy *et al.* 2008).

On the receptacle, at the base of the ovary and alternating with the filament of the stamens, 8 yellow protruding nectaries with the shape of a hook and organised in a circle secrete non-protected nectar (**Fig. 1G**). Nectar secretion begins after the opening of the perianth and nectar drops accumulate on the receptacle against the nectaries (Cawoy *et al.* 2008). The nectaries are made of a modified monolayered epidermis covering a multilayered nectary parenchyma (**Fig. 1H**; Cawoy *et al.* 2008). Nectar is secreted through epidermal unicellular trichomes located at the ventral part of the nectaries (**Fig. 1H**). The nectary parenchyma is supplied in water and nutrients by vascular bundles consisting in phloem and xylem connected to the vascular system of the other floral organs. A parenchyma with some chloroplasts constitutes the underlying tissue: the sub-nectary parenchyma.

The nectar is composed of sucrose, fructose and glucose (Kirillenko and Bochkareva 1983; Alekseyeva and Bureyko 2000; Cawoy *et al.* 2006a). The two hexoses (fructose and glucose) are the major components of buckwheat nectar (85%; nectar 'hexoses-dominant'; Cawoy *et al.* 2006a). Fructose becomes the main sugar with more than 50% at the flowering peak (Lee and Heimpel 2003). In the field, an average nectar production is estimated to 0.08-0.10 mg sugar per flower during the period of full flowering (Alekseyeva and Bureyko 2000). In controlled conditions, a flower products about 0.16 µl of nectar after 10 hours of secretion (Cawoy *et al.* 2006a). According to Lee and Heimpel (2003) sugar concentration in the field varies along the day: from 34% in early morning to less than 10% at mid-day. However, other total sugar concentrations are reported in the literature, fluctuating from 36 to 51% under field conditions and up to 55% under controlled conditions (Jablonski and Szklanowska 1990; Lee and Heimpel 2003; Racys and Montviliene 2005; Cawoy *et al.* 2006a). Differences in air moisture and secretion rate could be responsible for these fluctuations.

Biological characteristics of buckwheat cultivars influence nectar production (Naumkin 1998; Alekseyeva and Bureyko 2000). Tetraploid cultivars produce more nectar and pollen than diploids (30-40% more) and are thus more attractive for insects (Kirillenko 1984; Jablonski and Szklanowska 1990; Alekseyeva and Bureyko 2000). Although at the exception of the flowers both morphs are morphologically identical, thrum flowers produce until 30% more nectar than pin flowers during the first half part of the flowering (Cawoy *et al.* 2006a). Nevertheless, no differences in nectaries histology or in the sucrose supply to inflorescences were observed between the two floral morphs (Cawoy *et al.* 2008). The observed differences in nectar production between morphs in distylous species are unusual (Ornelas *et al.* 2004; Teixeira and Machado 2004). Total sugar concentration is similar in both morphs, but sucrose concentration is significantly higher in thrum flowers (16.8 *versus* 12.9%). Sucrose/hexoses ratio is therefore higher for thrum plants (Cawoy *et al.* 2006a).

Nectar production does not significantly differ between pollinated and unpollinated flowers and no nectar reabsorption was observed (Cawoy *et al*. 2006a, 2008). Nectar production of flowers is positively related to the number of open flowers per plant which fluctuates with the plant age (Cawoy *et al*. 2006a). The amount of nectar per flower and per plant is the highest during the flowering peak. Inflorescences of the terminal cluster have the maximum nectar production per flower (Cawoy *et al*. 2008).

Environmental conditions influence strongly nectar production and relative concentration of sugars (Naumkin 1998; Alekseyeva and Bureyko 2000). Light is required to induce nectar secretion. Transferring plants or inflorescences from light to darkness stops the nectar production. Under controlled conditions, nectar secretion is stable along the light period (Cawoy *et al*. 2008).

Volume of nectar per flower is light dependent as showed by the increasing of nectar secretion of 41% when the light irradiance doubles (Cawoy 2007). Nectar secretion appears thus linked to photosynthesis but, after a whole defoliation, despite the absence of the photosynthetic leaf area, the nectar secretion persists and the nectar is still highly sugared (defoliation: minimum 34%; control: 50%). Therefore, the photosynthesis may take place in other parts of the plant as the inflorescence pedicels, the cyme bracts and the main stem (Cawoy *et al*. 2008).

Low temperatures reduce sucrose production and modify consequently the relative concentration of sugars in buckwheat nectar (Alekseyeva and Bureyko 2000). With a cool and wet weather, sugar production per flower can be 15 times higher than during droughty periods (Racys and Montviliene 2005). Optimal soil humidity for nectar production averages 60% and water stress decreases sugar production (Alekseyeva and Bureyko 2000). Therefore sowing dates could affect nectar production (Naumkin 1998; Alekseyeva and Bureyko 2000). High soil fertility could also have a positive influence (Munitsa 1978).

POLLINATORS

Self-incompatibility of buckwheat requires cross-pollination between pin and thrum flowers (Nagatomo and Adachi 1985; Adachi 1990). Ren and Liu (1986) showed that pollen dispersal can reach 600 m if wind speed is lower than 3 m s^{-1} and 1000 m when wind speed is higher than 6 m s^{-1}. However, 1% of flowers are wind pollinated (Björkman 1995a). Therefore, insect pollination is the main pollination mechanism in buckwheat. It is visited by a diverse fauna, including *Hymenoptera*: honeybees (*Apis mellifera* L.), bumblebees, solitary bees and wasps; *Diptera*: *Syrphidae*, *Calliphoridae* and others; *Lepidoptera*, *Hemiptera*, *Nevroptera* and other orders (Ren and Liu 1986; Hedtke and Pritsch 1993; Ogasahara *et al.* 1995; Lee and Choi 1997; Naumkin 1998; Wang and Li 1998; Michiyama *et al.* 2000; Goodman *et al*. 2001; Carreck and Williams 2002; Racys and Montviliene 2005; Jacquemart *et al.* 2007). For example, 49 insect species, belonging to 18 families, were recorded in central Belgium (Jacquemart *et al.* 2007). In buckwheat fields, the most common visitors belong to *Apoidae* (*Hymenoptera*, *Apis mellifera* and *Bombus* spp.) and *Syrphidae* (*Diptera*, *Eristalis* spp.; Björkman 1995a; Wang and Li 1998; Carreck and Williams 2002; Racys and Montviliene 2005; Jacquemart *et al.* 2007).

Main visitor identity varies according to country, year, month and period of the day (Hedtke and Pritsch 1993; Björkman 1995a; Lee and Choi 1997; Jacquemart *et al.* 2007). In most countries where buckwheat is cultivated, honeybees are commonly considered as the main pollinators (McGregor 1976; Namai 1990a; Hedtke and Pritsch 1993; Björkman 1995a, 1995c; Lee and Choi 1997; Goodman *et al.* 2001; Jacquemart *et al.* 2007). Accordingly, apiarists recommend introduction of bee-hives to ensure satisfactory seed yield (McGregor 1976; Wang and Li 1998; Dalby 2000; Goodman *et al.* 2001). However, honeybees represent only 2-5% of the total floral visitors in Japan, 35% in China and 37% in Russia (McGregor 1976; Ogasahara *et al.* 1995; Wang and Li 1998; Sasaki and Wagatsuma 2007). Around its area of origin (China), other *Hymenoptera* (*Bombus*, *Andrena*, *Osmia* and *Megachile*) represent 25% and *Syrphidae* 27% of the floral visitors (Wang and Li 1998). Variation in the visitor guild occurs in Belgium between the beginning and the end of the flowering period, as the proportion of honeybee was higher in July (50%) than in September (21%; Jacquemart *et al.* 2007). This decrease in honeybee abundance at the end of the summer could be linked to the decrease of nectar production observed after the flowering peak (Naumkin 1998; Alekseyeva and Bureyko 2000; Cawoy *et al.* 2006a). Variations of buckwheat visitor guild between months have already been reported elsewhere (Lee and Choi 1997; Carreck and Williams 2002). Bumblebees and honeybees visit buckwheat predominantly between 09.00-12.00 h daily whereas syrphids are still active in the afternoon (McGregor 1976; Free 1993; Hedtke and Pritsch 1993; Limonta and Antignati 1994; Naumkin 1998; Alekseyeva and Bureyko 2000; Goodman *et al.* 2001; Jacquemart *et al.* 2007).

POLLINATION

Pollination efficiency depends on the insect abundance and on the insect ability to collect, transport and deposit pollen on a compatible stigma.

Honeybee foraging activity (time spent per inflorescence and plant) is not affected by day period, although fewer flowers are visited per trip after mid-day, whereas syrphid foraging activity decreases in the course of the day (Jacquemart *et al.* 2007). Honeybees are more active during warm and sunny days as well as during the flowering peak (Alekseyeva and Bureyko 2000). A single honeybee visits an average of 14-20 flowers min^{-1}, and works on buckwheat for 4-5 h d^{-1} (Free 1993; Hedtke and Pritsch 1993; Jacquemart *et al.* 2007). This insect appears to be the most effective pollinator of buckwheat because it collects both types of pollen (pin and thrum) on a same trip and its foraging and prospecting behaviour, collecting nectar and pollen, promotes frequent contacts with stigmas (**Fig. 1F**; Björkman 1995a; Jacquemart *et al.* 2007). Nevertheless, when more attractive flowers are available (as *Phacelia tanacetifolia*, *Raphanus sativus* and *Sinapis alba*) honeybees neglect buckwheat (Becker and Hedtke 1995; Hedtke 1996; Carreck and Williams 2002). Some syrphids (*Eristalis* spp.) and perhaps other *Diptera* species could act as co-pollinators due to their high relative frequency and activity during the entire flowering period (Namai 1990a; Alekseyeva and Bureyko 2000; Jacquemart *et al.* 2007).

Under experimental cages, the efficiency of honeybee pollination (introduced colony) is good since this insect deposits compatible pollen on most flowers (>90%) without discrimination between floral morphs (Jacquemart *et al.* 2007). On the other hand, Cawoy *et al.* (2006a) reported that honeybee spends more time on thrum (higher producer of nectar) than on pin flowers, particularly in the morning, visiting more thrum inflorescences and more thrum flowers per inflorescence. Despite its better pollinator attraction, thrum flower stigmas capture fewer pollen grains than those of pin flowers (Namai 1990a, syrphid; Björkman 1995b, *Apis mellifera*; Cawoy *et al.* 2006a, *Apis mellifera*). Nevertheless, according to Björkman (1995b), thrum flowers receive three times more compatible pollen grains than pin flowers. However, Cawoy *et al.* (2006b) observed a similar number of pollen tubes in the style of both morphs.

Along the day, pollen deposition on stigmas and pollen tube growth increase. The saturating pollen load for fruit initiation occurs at about 10 pollen grains per flower allowing a high fruit set (80-90%; Namai and Ohsawa 1986; Björkman 1995c). Seed weight and progeny vigor increase if pollen number is higher than 10 grains due to gametophytic competition (Skerbtosova *et al.* 1974 *in* Namai 1990a; Björkman 1995c). A single insect visit delivers only an average of 5 pollen grains (Namai 1990a; Björkman 1995c). Two or more honeybee visits are therefore required for an optimal fruit set, similar to hand cross pollination (Björkman 1995a). However, the most flowers mainly pollinated by honeybees in the Belgian fields present less than 10 pollen grains at the end of their life (one single day; Cawoy *et al.* 2006a). Moreover, more than one hour usually separated two successive insect visits. This delay limits gametophytic competition as pollen tube growth is very fast: only 5-10 min are necessary to reach the ovules in thrum styles and 15-20 min in pin styles (Schock-Bodmer 1930; Tatebe 1956; Campbell 1997). These facts could be seemed as a pollen limitation transfer, but although seed set is low (about 15%) under field conditions in Belgium, it does not increase after hand cross-pollination, suggesting an absence of pollen limitation (Cawoy *et al.* 2006a). These results show that factors other than pollination events are leading fertility in buckwheat. Nevertheless, in order to avoid insufficient pollination, the availability of pollinators, such as honeybees which are the main pollinators of buckwheat in Western Europe, should be considered before choosing field location or to add hives.

FUNCTIONALITY OF FLOWERS

Even when possible effect of an inadequate pollination by insects is eliminated through hand pollination with exclusively compatible pollen, a variable proportion (35-97%) of the flowers does not set seed (Björkman 1995c; Taylor and Obendorf 2001; Cawoy *et al.* 2007). In experiment without pollen limitations, Taylor and Obendorf (2001) showed that 76 to 91% of the pollinated flowers did not show any sign of fertilization. Three causes can explain the seed production failure: male or female sterility or/and embryo abortion.

Male sterility seems a marginal event since studies on pollen germination show a good viability (>90%; Hirose *et al.* 1994; Adhikari and Campbell 1998; Cawoy *et al.* 2006b; Chen *et al.* 2007). Björkman (1995c) estimates that flowers pollinated with at least 90 grains of pollen and that are not producing seed have a deficiency of female reproductive structures.

Female deficiencies of flowers depend on their location on the plant and on the plant age (Halbrecq *et al.* 2005; Cawoy *et al.* 2007). Flowers have not the same probability to initiate a seed within an inflorescence. Seed set decreases from the basis to the summit where seed set becomes null (Halbrecq *et al.* 2005; Cawoy *et al.* 2007). First flowers in anthesis are also more likely to produce a seed than the following ones (Asako *et al.* 1980; Björkman *et al.* 1995c; Taylor and Obendorf 2001). Samborborska-Ciana *et al.* (1989a) observed a decrease in the size of gynoecia through time and Nagatomo and Adachi (1985) mentioned the presence of numerous flowers with pistil of abnormal small size. According to Cawoy *et al.* (2007), occurrence of undersized and sterile gynoecia is dependent on the position of the flower in the raceme – being more frequent in distal than in proximal cymes (until 49% of flowers in the last third of an inflorescence) – and increases when seeds are developing in the inflorescence (increase of 26%). In controlled conditions, among the morphologically normal hand pollinated flowers, less than 10% of the flowers exhibited a later abortion of seed (i.e. ovary expended) and about 60% did not develop seed (Cawoy *et al.* 2007). "Early abortion" (i.e. wilted flower) observed just after flowering mainly accounts for the low final yield in buckwheat (Taylor and Obendorf 2001; Halbrecq *et al.* 2005; Cawoy *et al.* 2007). Taylor and Obendorf (2001) attempted to make the difference between early abortion of embryo and sterility of female organs. Early abortion of embryo (24 h after pollination) involved on average 10% of pollinated flowers and therefore was not the most important limiting factor for yield. Flowers presenting a non-viable mega-gametophyte at anthesis represented 20% and their proportion remained constant during the flowering period. Nevertheless, 30% of flowers at the beginning and 60% at the end of flowering presented a normal embryo sac without fertilization 24 h after pollination. According to Taylor and Obendorf (2001), this could be explained by a wrong alignment of the canal of the style and the micropyle. The occurrence of this aberration, which is dependent of the flower position in the raceme similarly as the occurrence of undersized gynoecia, suggests that it could result from an alteration of flower morphogenesis, in response to a limitation of resource availability (Gang and Yu 1998; Halbrecq *et al.* 2005; Cawoy *et al.* 2007). These variations in the female reproductive success in an inflorescence could be induced by a resource competition among structures (vegetative organs, meristems producing flowers, young reproducing organs and fruits in formation) and/or a limited resource supplying inherent to the inflorescence and resulting of this structure (i.e. distal flower are lesser supplied in resource than proximal ones because the diameter of the sieve-tube cells decreases; see review *in* Cawoy *et al.* 2007). However, a mechanism own to the raceme seems to regulate flower fate in an inflorescence independently of assimilate availability (Cawoy *et al.* 2007). When applied during the inflorescence morphogenesis, treatments altering source/sink organ ratio by excision of selected inflorescences enhances the number of flowers. Compensation for the loss of racemes is partial: the inflorescences that are kept produced an increased number of flowers that are insufficient to balance the loss due to the excisions. Despite the increase of resource availability, treatments do not change the final distribution between wilted pollinated flowers with a normal sized gynoecium, flowers with an undersized gynoecium, aborted seeds and ripe seeds (Cawoy *et al.* 2007). The fate of flowers appears therefore to be dependent on a mechanism internal to the raceme which controls the percentage of flowers able to set a seed (Cawoy *et al.* 2007). This mechanism may be disturbed by drastic reductions of resource supply (e.g. a large defoliation).

CONCLUSION

Due to the sensitivity of buckwheat to climatic factors, sowing date strongly influences the yield of this crop (Baumgärtner *et al.* 1998; Inoue *et al.* 1998; Jessop *et al.* 1998; Halbrecq *et al.* 2005; Cawoy *et al.* 2006b). Pollinator abundance, mainly honeybees, is also an important factor that guarantees the achene yield (Ren and Liu 1986; Jablonski and Szklanowska 1990; Björkman 1995a; Lee and Choi 1997; Wang and Li 1998; Goodman *et al.* 2001; Cawoy *et al.* 2006b; Jacquemart *et al.* 2007). Therefore, agronomical practices could be locally improved to optimise seed set of buckwheat.

Resource availability influences the reproductive morphogenesis and consequently the number of flowers produced by plants and the duration of the flowering period. On the other hand, the fate of flowers appears to be dependent on a mechanism internal to the raceme which controls the percentage of flowers able to set a seed (Cawoy *et al.* 2007). Flowers with a normal sized gynoecium which do not set seed after compatible pollination are the main contribution to the low seed set in buckwheat. This female organ sterility seems to be the main yield limiting factor of common buckwheat throughout the world. However, its origin still remains unknown. Understanding of mechanisms which control this sterility may help to improve significantly yields.

ACKNOWLEDGEMENTS

The National Fund for Scientific Research in Belgium funded our researches (FRFC n° 2.4562.99 and n° 2.4.59704.F). All our thanks to V. Deblauwe, V. Droissart, C. Gillet, B. Halbrecq, S. Lutts and M. Quinet for their field and lab assistance and to two anonymous reviewers whose comments have greatly improved the manuscript.

REFERENCES

Adachi T (1990) How to combine the reproductive system with biotechnology in order to overcome the breeding barrier in buckwheat. *Fagopyrum* **10**, 7-11

Adachi T, Kawabata K, Matsuzaki N, Yabuya T, Nagatomo T (1983) Observation of pollen tube elongation, fertilization and ovule development in autogamous autotetraploid buckwheat. In: Nagatomo T, Adachi T (Eds) *Buckwheat Research, Proceedings of the 2nd International Symposium on Buckwheat*, 7-10 September 1983, Miyazaki, Kuroda-toshado Printing, Miyazaki, Japan, pp 103-113

Adhikari KN, Campbell CG (1998) *In vitro* germination and viability of buckwheat (*Fagopyrum esculentum* Moench) pollen. *Euphytica* **102**, 87-92

Aii J, Nagano M, Penner GA, Campbell CG, Adachi T (1998) Identification of RAPD markers linked to the homostylar (*Ho*) gene in buckwheat. *Breeding Science* **48**, 59-62

Alekseyeva ES, Bureyko AL (2000) Bee visitation, nectar productivity and pollen efficiency of common buckwheat. *Fagopyrum* **17**, 77-80

Asako Y, Ujihara A, Matano T (1980) Relation between the position of flowers and their flowering of fruiting in common buckwhat. *Hokuriku Crop Science* **15**, 27-30

Bahadur B, Laxmi SB, Swamy NR (1984) Pollen morphology and heterostyly. A systematic and critical account. *Advances in Pollen Spore Research* **12**, 79-129

Baumgärder J, Schilperoord P, Basetti P, Baiocchi A, Jermini M (1998) The use of a phenology model and of risk analyses for planning buckwheat (*Fagopyrum esculentum*) sowing dates in alpine areas. *Agricultural Systems* **57**, 557-569

Becker K, Hedtke C (1995) Foraging of wild bees and honey bees on a mixture of entomophilous plants on extensification areas (fallow land). *Apidologie* **26**, 344-346

Björkman T (1995a) The role of honeybees (Hymenoptera: Apidae) in the pollination of buckwheat in eastern North America. *Journal of Economic Entomology* **88**, 1739-1745

Björkman T (1995b) The effectiveness of heterostyly in preventing illegitimate pollination in dish-shaped flowers. *Sexual Plant Reproduction* **8**, 143-146

Björkman T (1995c) The effect of pollen load and pollen grain competition on fertilization success and progeny performance in *Fagopyrum esculentum. Euphytica* **83**, 47-52

Björkman T (2000) Buckwheat production. Guide to buckwheat production in the Northeast. Available online: http:/www.nysaes.cornell.edu/hort/faculty/bjorkman/buck/Buck.html

Bonafaccia G, Gambelli L, Fabjan N, Kreft I (2003a) Trace elements in flour and bran from common and tartary buckwheat. *Food Chemistry* **83**, 1-5

Bonafaccia G, Marocchini M, Kreft I (2003b) Composition and technological properties of the flour and bran from common and tartary buckwheat. *Food Chemistry* **80**, 9-15

Campbell CG (1997) Buckwheat: *Fagopyrum esculentum* Moench. Promoting the conservation and use of underutilized and neglected crops, 19. International Plant Genetic Resources Institute, Rome, Italy, 95 pp

Carreck NL, Williams IH (2002) Food for insect pollinators on farmland: insect visits to flowers of annual seed mixtures. *Journal of Insect Conservation* **6**, 13-21

Cawoy V (2007) Etude du contrôle de la floraison et de la fructification chez le sarrasin commun, *Fagopyrum esculentum* Moench (cv. La Harpe). PhD thesis, Université Catholique de Louvain, Belgium, 252 pp

Cawoy V, Deblauwe V, Halbrecq B, Ledent J-F, Kinet J-M, Jacquemart A-L (2006a) Morph differences and honeybee morph preference in the distylous species *Fagopyrum esculentum* Moench. *International Journal of Plant Sciences* **167**, 853-861

Cawoy V, Kinet J-M, Jacquemart A-L (2008) Morphology of nectaries and biology of nectar production in the distylous species *Fagopyrum esculentum* Moench. *Annals of Botany* **102**, 675-684

Cawoy V, Lutts S, Kinet J-M (2006b) Osmotic stress at seedling stage impairs reproductive development in buckwheat (*Fagopyrum esculentum*). *Physiologia Plantarum* **128**, 689-700

Cawoy V, Lutts S, Ledent J-F, Kinet J-M (2007) Resource availability regulates reproductive meristem activity, development of reproductive structures and seed set in buckwheat (*Fagopyrum esculentum* Moench). *Physiologia Plantarum* **131**, 341-353

Chen QF, Hsam SLK, Zeller FJ (2004) A study of cytology, isozyme, and interspecific hybridization on the big-achene group of buckwheat species (*Fagopyrum*, Polygonaceae). *Crop Science* **44**, 1511-1518

Chen QF, Hsam SLK, Zeller FJ (2007) Cytogenetic studies on diploid and autotetraploid common buckwheat and their autotriploid and trisomics. *Crop Science* **47**, 2340-2345

Christa K, Soral-Śmietana M (2008) Buckwheat grains and buckwheat products - nutritional and prophylactic value of their components - a review. *Czech Journal of Food Sciences* **26**, 153-162

Dalby R (2000) Classic American honey plants: buckwheat. *American Bee Journal* **140**, 485-486

FAOSTAT data (2006) Online database. Available online: http://faostat.fao.org

Free JB (1993) *Insect Pollination of Crops*, Academic Press, London, UK, 544 pp

Funatsuki H, Maruyama-Funatsuki W, Fujino K, Agatsuma M (2000) Ripening habit of buckwheat. *Crop Science* **40**, 1103-1108

Ganders FR (1979) The biology of heterostyly. *New Zealand Journal of Botany* **17**, 607-635

Gang Z, Yu T (1998) A primary study of increasing the production rate of buckwheat. In: Campbell C, Przybylski R (Eds) *Current Advances in Buckwheat Research (Vol II), Proceedings of the 7th International Symposium on Buckwheat*, 12-14 August 1998, Winnipeg, Manitoba, Canada, pp 18-23

Goodman R, Hepworth G, Kaczynski P, McKee B, Clarke S, Bluett C (2001) Honeybee pollination of buckwheat (*Fagopyrum esculentum* Moench) cv. Manor. *Australian Journal of Experimental Agriculture* **41**, 1217-1221

Guan LM, Adachi T (1992) Reproductive deterioration in buckwheat (*Fagopyrum esculentum*) under summer conditions. *Plant Breeding* **109**, 304-312

Guan LM, Adachi T (1994) Ultrastructural changes of the mature embryo sac in buckwheat (*Fagopyrum esculentum*) as a result of high temperature exposure. *Cytologia* **59**, 237-248

Hagels H (1999) *Fagopyrum esculentum* Moench. Medicinal review. *Research Reports, Biotechnical Faculty, University of Ljubljana (Slovenia)* **73**, 315-329

Hagiwara M, Inoue N, Matano T (1998) Variability in the length of flower bud differentiation period of common buckwheat. *Fagopyrum* **15**, 55-64

Halbrecq B, Romedenne P, Ledent JF (2005) Evolution of flowering, ripening and seed set in buckwheat (*Fagopyrum esculentum* Moench): quantitative analysis. *European Journal of Agronomy* **23**, 209-224

Hao X, Li G, Yang W, Zhou N, Lin R, Zhou M (1995) The difference and classification of light reaction of buckwheat under different treatments of light duration. In: Matano T, Ujihara A (Eds) *Current Advances in Buckwheat Research, Proceedings of the 6th International Symposium on Buckwheat*, 24-29 August 1995, Shinshu, Shinshu University Press, Shinshu, Japan, pp 541-549

Hedtke C (1996) The attractiveness of 5 cultivated crops to bees and a mixture of annual plants for flower visiting insects. *Pszcelnicze Zeszyty Naukowe* **40**, 219-225

Hedtke C, Pritsch G (1993) Qualitative and quantitative investigation of insects foraging on buckwheat (*Fagopyrum esculentum* Moench). *Apidologie* **24**, 476-477

Hirose T, Ujihara A, Kitabayashi H, Minami M (1994) Interspecific cross-compatibility in *Fagopyrum* according to pollen tube growth. *Breeding Science* **44**, 307-314

Inoue N, Hagiwara M, Kim HY, Matano T (1998) A preliminary study for modelling seed production in common buckwheat. *Fagopyrum* **15**, 35-41

Jablonski B, Szklanowska K (1990) Beekeeping value and pollination requirements of tetraploid buckwheat. *Pszczelnicze Zeszyty Naukowe* **34**, 51-56

Jacquemart A-L, Gillet C, Cawoy V (2007) Floral visitors and importance of honey bee on buckwheat (*Fagopyrum esculentum* Moench) in central Belgium. *The Journal of Horticultural Science and Biotechnology* **82**, 104-108

Jessop RS, Clark DA, Williams S (1998) Factors affecting flowering efficiency in Australian buckwheat. In: Campbell C, Przybylski R (Eds) *Current Advances in Buckwheat Research (Vol II), Proceedings of the 7th International Symposium on Buckwheat*, 12-14 August 1998, Winnipeg, Manitoba, Canada, pp 92-95

Kalinová J, Moudrý J (2003) Evaluation of frost resistance in varieties of common buckwheat (*Fagopyrum esculentum* Moench). *Plant Soil and Environment* **49**, 410-413

Kalinová J, Moudrý J, Čurn V (2002) Technological quality of common buckwheat (*Fagopyrum esculentum* Moench). *Rostlinná Výroba* **48**, 279-284

Kawa JM, Taylor CG, Przybylski R (2003) Buckwheat concentrate reduces serum glucose in streptozotocin-diabetic rats. *Journal of Agricultural and Food Chemistry* **51**, 7287-7291

Kim SL, Kim SK, Park CH (2004) Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable. *Food Research International* **37**, 319-327

Kirillenko SK (1984) Nectar production in buckwheat. *Pchelovodstvo* **6**, 17-18

Kirillenko SK, Bochkareva LP (1983) Nectar yield in buckwheat flowers. *Pchelovodstvo* **6**, 12-13

Komenda K, Komenda-Ronka J, Komenda B (1986) Study of buckwheat varieties collection (*Fagopyrum* ssp.). In: Institute of Soil Science and Plant Cultivation (Ed) *Buckwheat Research 1986, Proceedings of the 3rd International Symposium on Buckwheat* (Vol 2), Laboratory of Science, Pulawy, Poland, pp 68-70

Kreft I (1989) Breeding of determinate buckwheat. *Fagopyrum* **9**, 57-59

Krkošková B, Mrázová Z (2005) Prophylactic components of buckwheat. *Food Research International* **38**, 561-568

Lachmann S, Adachi T (1990) Studies of the influence of photoperiod and temperature on floral traits in buckwheat *Fagopyrum esculentum* Moench under controlled stress conditions. *Plant Breeding* **105**, 248-253

Lakhanov AP (1991) Efficiency of water consumption by buckwheat varieties with varying water supply to plants in southern nonchernozem zone of RSFSR. *Doklady Vsesoyuznoi Akademii Sel'skokhozyaistvennykh Nauk* **10**, 5-8

Lakhanov AP, Napolova GV (2001) Comparative studies on morpho-physiological characteristics of plants between wild species of buckwheat. In: Ham SS, Choi YS, Kim NS, Park CH (Eds) *Currents Advances in Buckwheat Research, Proceedings of the 8th International Symposium on Buckwheat*, August 30-September 2 2001, Chunchon, Republic of Korea, pp 3-9

Lee HR, Choi MH (1997) Studies on foraging activities, efficacies of the pollinators and pollen characteristics of Korean raspberry (*Rubus crataegifolius*), buckwheat (*Fagopyrum esculentum*) and Japanese Cornelian cherry (*Cornus officinalis*). *Korean Journal of Apiculture* **12**, 69-76

Lee JC, Heimpel GE (2003) Nectar availability and parasitoid sugar feeding. In: Van Driesche RG (Ed) *Proceedings of the 1st International Symposium on the Biological Control of Arthropods*, 14-18 January 2002, Honolulu, Hawaii, USA, Department of Agriculture Forest Service, Morgantown, WV, USA, pp 220-225

Lee MS (1986) Studies on heterostylism, fertility, and embryological characteristics in buckwheat, *Fagopyrum esculentum*. *Korean Journal of Crop Science* **31**, 129-142

Lewis D, Jones DA (1992) The genetics of heterostyly. In: Barrett SCH (Ed) *Evolution and function of heterostyly*, Springer-Verlag, Berlin, Germany, pp 129-148

Limonta L, Antignati E (1994) Researches on the main pollinating insects of buckwheat (*Fagopyrum esculentum* Moench) in Valtellina. *Apicoltore Moderno* **85**, 5-12

Liu CL, Chen YS, Yang JH, Chiang BH (2008) Antioxidant activity of tartary (*Fagopyrum tataricum* (L.) Gaertn.) and common (*Fagopyrum esculentum* Moench) buckwheat sprouts. *Journal of Agricultural and Food Chemistry* **56**, 173-178

Logacheva MD, Samigullin TH, Dhingra A, Penin AA (2008) Comparative chloroplast genomics and phylogenetics of *Fagopyrum esculentum* ssp *ancestrale* - A wild ancestor of cultivated buckwheat. *BMC Plant Biology* **8**, art n° 59

Marshall HG, Pomeranz Y (1982) Buckwheat: description, breeding, production and utilization. In: Pomeranz Y (Ed) *Advances in Cereal Science and Technology* (Vol V), American Association of Cereal Chemist Incorporated Inc., St Paul, Minnesota, USA, pp 157-210

Matsui K, Nishio T, Tetsuka T (2004) Genes outside the *S* supergene suppress S functions in buckwheat (*Fagopyrum esculentum*). *Annals of Botany* **94**, 805-809

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

McGregor SE (1976) *Buckwheat*. Insect pollination of cultivated crop plants. Agricultural Handbook No. 496. Agricultural Research Service, United States Department of Agriculture, USA. Available online:
http://gears.tucson.ars.ag.gov/book/chap9/buckwheat.html

Michiyama H, Arikuni M, Hirano T, Hayashi H (2003) Influence of day length before and after the start of anthesis on the growth, flowering and seed-setting in common buckwheat (*Fagopyrum esculentum* Moench). *Plant Production Science* **6**, 235-242

Michiyama H, Fukui A, Hayashi H (1998) Differences in the progression of succesive flowering between summer and autumn ecotype cultivars in common buckwheat (*Fagopyrum esculentum* Moench). *Japanese Journal of Crop Science* **67**, 498-504

Michiyama H, Miyabara N, Arikuni M, Hirano T (2000) Effect of artificial pollination and exclusion of pollinating insects on flowering and seed-setting in common buckwheat (*Fagopyrum esculentum* Moench). *Report of the Tokai Branch of the Crop Science Society of Japan* **129**, 9-17

Miljuš-Đukić J, Ninković S, Nešković (2003) Effects of protein phosphatase inhibitors and calcium antagonists on self-incompatible reaction in buckwheat. *Biologia Plantarum* **46**, 475-478

Miljuš-Đukić J, Ninković S, Radović S, Maksimović V, Brkljačić J, Nešković M (2004) Detection of proteins possibly involved in self-incompatibility response in distylous buckwheat. *Biologia Plantarum* **48**, 293-296

Minami H, Namai H (1986) Populational change in flowering time caused by different harvesting date observed in a late-summer type cultivar Miyazaki-zairai of buckwheat *Fagopyrum esculentum*. *Japanese Journal of Breeding* **36**, 155-162

Munitsa MY (1978) Effect of complex fertilizers with trace elements on the yield, nectar-yield and quality of buckwheat. Nauchnye Trudy. *Ukrainskaya Sel'skokhozyaistvennaya Akademiya* **205**, 84-86

Murakami T, Murayama S, Uchitsu M, Yoshida S (2002) Root length and distribution of field-grown buckwheat (*Fagopyrum esculentum* Moench). *Soil Science and Plant Nutrition* **48**, 609-613

Nagano M, Aii J, Kuroda M, Campbell C, Adachi T (2001) Conversion of AFLP markers linked to the S^h allele at the *S* locus in buckwheat to a simple PCR based marker form. *Plant Biotechnology* **18**, 191-196

Nagatomo T, Adachi T (1985) *Fagopyrum esculentum*. In: Halevy AH (Ed) *Handbook of Flowering* (Vol III), CRC Press, Boca Raton, FL, USA, pp 1-8

Namai H (1990a) Pollination biology and reproductive ecology for improving genetics and breeding of common buckwheat, *Fagopyrum esculentum* (1). *Fagopyrum* **10**, 23-46

Namai H (1990b) Pollination biology and reproductive ecology for improving genetics and breeding of common buckwheat, *Fagopyrum esculentum* (2). *Fagopyrum* **11**, 35-46

Namai H, Fujita Y (1995) Floral characteristics associated with seed productivity in common buckwheat, *Fagopyrum esculentum* Moench. In: Matano T, Ujihara A (Eds) *Current Advances in Buckwheat Research, Proceedings of the 6th International Symposium on Buckwheat*, 24-29 August 1995, Shinshu, Shinshu University Press, Shinshu, Japan, pp 425-435

Namai H, Ohsawa R (1986) Variation of reproductive success rates of ovule and pollen deposited upon stigmas according to the different number of pollen on a stigma in angiosperm. In: Mulcahy DL, Mulcahy GB, Ottaviano E (Eds) *Biotechnology and Ecology of Pollen*, Springer-Verlag, New York, USA, pp 423-428

Naumkin VP (1998) Increase of productivity of nectar and yield of buckwheat via flower-nectar flow creation. In: Campbell C, Przybylski R (Eds) *Current Advances in Buckwheat Research (Vol III), Proceedings of the 7th International Symposium on Buckwheat*, Canada, 12-14 August 1998, Winnipeg, Manitoba, Canada, pp 1-5

Obendorf RL, Horbowicz M, Taylor DP (1993) Structure and chemical composition of developing buckwheat seed. In: Janick J, Simon JE (Eds) *New Crops*, John Wiley and Sons, New York, USA, 244-251

Ogasahara S, Kaji C, Hagiwara M, Matamo T (1995) Pollination of common buckwheat (*Fagopyrum esculentum* Moench) as influenced by meteorological conditions. In: Matano T, Ujihara A (Eds) *Current Advances in Buckwheat Research, Proceedings of the 6th International Symposium on Buckwheat*, 24-29 August 1995, Shinshu, Shinshu University Press, Shinshu, Japan, pp 475-481

Ornelas JF, Gonzáles C, Jiménez L, Lara C, Martinez AJ (2004) Reproductive ecology of distylous *Palicourea padifolia* (Rubiaceae) in a tropical montane cloud forest. II. Attracting and rewarding mutualistic and antagonistic visitors. *American Journal of Botany* **91**, 1061-1069

Quinet M, Cawoy V, Lefèvre I, Van Miegroet F, Jacquemart A-L, Kinet J-M (2004) Inflorescence structure and control of flowering time and duration by light in buckwheat (*Fagopyrum esculentum* Moench). *Journal of Experimental Botany* **55**, 1509-1517

Racys J, Montvilienne R (2005) Effect of bee-pollinators in buckwheat (*Fagopyrum esculentum* Moench) crops. *Journal of Apicultural Science* **49**, 47-51

Ren SH, Liu AL (1986) The survey of cultivated buckwheat, pollen spreading distance and the relation between pollen carried by insects and yield. In: Institute of Soil Science and Plant Cultivation (Ed) *Buckwheat Research 1986 (Part I), Proceedings of the 3rd International Symposium on Buckwheat*, Laboratory of Science Publisher, Pulawy, Poland, pp 10-21

Samborska-Ciania A, Januszewicz E, Ojczyk T (1989a) The morphology of buckwheat flowers depending on the course of plant flowering. *Fagopyrum* **9**, 23-26

Samborska-Ciania A, Januszewicz E, Ojczyk T (1989b) The variation of traits related to heterostyly of buckwheat (*Fagopyrum esculentum* Moench) flowers. *Fagopyrum* **9**, 27-30

Sasaki H, Wagatsuma T (2007) Bumblebees (Apidae: Hymenoptera) are the main pollinators of common buckwheat, *Fagopyrum esculentum*, in Hokkaido, Japan. *Applied Entomology and Zoology* **42**, 659-661

Schoch-Bodmer H (1930) Zur Heterostylie von *Fagopyrum esculentum*: Untersuchungen über das Pollenschlauchwachstum und über die Saugkräfte der Griffel und Pollenkörner. *Schweizerische Botanische Gessellschaft Berichte* **39**, 4-15

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

Sharma KD, Boyes JM (1961) Modified incompatibility of buckwheat following radiations. *Canadian Journal of Botany* **39**, 1241-1246

Slawinska J, Obendorf RL (2001) Buckwheat seed set *in planta* and during *in vitro* inflorescence culture: evaluation of temperature and water deficit stress. *Seed Science Research* **11**, 223-233

Sugimoto H, Sato T (2000) Effects of excessive soil moisture at different growth stages on seed yield of summer buckwheat. *Japanese Journal of Crop Science* **69**, 189-193

Tahir I, Farooq S (1988) Review article on buckwheat. *Fagopyrum* **8**, 33-53

Tang CH, Peng J, Zhen DW, Chen, Z (2009) Physicochemical and antioxidant properties of buckwheat (*Fagopyrum esculentum* Moench) protein hydrolysates. *Food Chemistry* **115**, 672-678

Tatebe T (1956) Physiological researches on the fertility of the buckwheat. (V) Further studies on the behaviour of pollen and pollen-tube growth. *Japanese Journal of Breeding* **6**, 156-162

Taylor DP, Obendorf RL (2001) Quantitative assessment of some factors limiting seed set in buckwheat. *Crop Science* **41**, 1792-1799

Teixeira LAG, Machado IC (2004) Pollination biology and breeding system of *Psychotria barbiflora* DC (Rubiaceae). *Acta Botanica Brasilica* **18**, 853-862

Tomotake H, Shimaoka I, Katashita J, Yokoyama F, Nakajoh M, Kato N (2000) A buckwheat protein product suppresses gallstone formation and plasma cholesterol more strongly than soy protein isolate in hamster. *Nutrition Journal* **130**, 1670-1674

Wagatsuma T (2004) Selection of determinate type plants from common buckwheat cultivar 'Kitawasesoba' and development of a new line, Horokei 3. *Journal of Rakuno Gakuen University, Natural Science* **29**, 1-7

Wang R, Li C (1998) Insect pollinators and yield of common buckwheat. In: Campbell C, Przybylski R (Eds) *Current Advances in Buckwheat Research (Vol II), Proceedings of the 7th International Symposium on Buckwheat*, Canada, 12-14 August 1998, Winnipeg, Manitoba, pp 18-23

Wang YJ, Scarth R, Campbell C (2005) S^h and S^c - two complementary dominant genes that control self-compatibility in buckwheat. *Crop Science* **45**, 1229-1234

Woo SH, Adachi T, Jong SK, Campbell CG (1999) Inheritance of self-compatibility and flower morphology in an inter-specific buckwheat hybrid. *Canadian Journal of Plant Science* **79**, 483-490

Yasui Y, Wang Y, Ohnishi O, Campbell CG (2004) Amplified fragment length polymorphism linkage analysis of common buckwheat (*Fagopyrum esculentum*) and its wild self-pollinated relative *Fagopyrum homotropicum*. *Genome* **47**, 345-351