

Inferring vascular architecture of the wheat spikelet based on resource allocation in the *branched head^t* (*bh^t-A1*) near isogenic lines

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Abstract. Substantial genetic and physiological efforts were made to understand the causal factors of floral abortion and grain filling problem in wheat. However, the vascular architecture during wheat spikelet development is surprisingly under-researched. We used the *branched head^t* near-isogenic lines, FL-*bh^t-A1*-NILs, to visualise the dynamics of spikelet fertility and dry matter accumulation in spikelets sharing the same rachis node (henceforth Primary Spikelet, PSt, and Secondary Spikelet, SSt). The experiment was conducted after grouping FL-*bh^t-A1*-NILs into two groups, where tillers were consistently removed from one group. Our results show differential spikelet fertility and dry matter accumulation between the PSt and SSt, but also showed a concomitant improvement after de-tillering. This suggests a tight regulation of assimilate supply and dry matter accumulation in wheat spikelets. Since PSt and SSt share the same rachis node, the main vascular bundle in the rachis/rachilla is expected to bifurcate to connect each spikelet/floret to the vascular system. We postulate that the vascular structure in the wheat spikelet might even follow Murray's law, where the wide conduits assigned at the base of the spikelet feed the narrower conduits of the distal florets. We discuss our results based on the two modalities of the vascular network systems in plants.

Additional keywords: assimilate partitioning, bifurcation, modeling, aorta, primary spikelet, secondary spikelet, spikelet fertility, *Triticum durum*, vascular bundle.

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Introduction

Wheat (*Triticum* spp.) is the most widely grown crop in the world as a direct source for human calorie consumption (Shiferaw *et al.* 2013; Shewry and Hey 2015). The source of the calories – the grain – is produced on a sessile specialised branch, called the spikelet, which contains a collection of grain-producing florets that are arranged on the main axis of the spikelet called the rachilla. Grain number in wheat is therefore strongly influenced by the number and arrangement of spikelets (Boden *et al.* 2015; Dobrovolskaya *et al.* 2015; Poursarebani *et al.* 2015; Dixon *et al.* 2018; Wolde *et al.* 2019a), as well as the fertility of the spikelet (Guo *et al.* 2017, 2018; Prieto *et al.* 2018; Sakuma *et al.* 2019). Hence, the simultaneous increase in spikelet number and spikelet fertility is important for increasing grain yield in wheat. Despite the fact that more floret primordium are developed indeterminately in the wheat spikelet (a maximum of 8–12 floret primordia initials from each mid-central spikelet), less than half of these are fertile at anthesis due to floret abortion and/or insufficient development (Kirby 1974, 1988; Whingwiri

and Stern 1982; Kirby and Appleyard 1984; McMaster 1997; Guo *et al.* 2017). In this regard, three hypotheses were previously suggested as a probable cause of floret abortion in wheat (Whingwiri and Stern 1982; McMaster 1997): (i) inadequate supply of mineral nutrients, water, and carbohydrates; (ii) hormonal imbalances; and (iii) a problem related to vascular development.

Since degeneration of floral primordia in wheat proceeds in a basipetal order, i.e. starts from the apex and proceeds to the base of the rachilla, only few of those florets at the base of the rachilla reach anthesis and set grains. In line with this, Hanif and Langer (1972) suggested that further development of the distal floral primordia is dependent on the development and anatomical structure of the vascular system. This suggests that a detailed understanding of the vascular bundle development and connectivity is essential to better understand floret abortion in wheat. Surprisingly, very few studies have attempted to explore and formulate the vascular structure of the wheat spikelet.

Whingwiri and Stern studied the vascular system in the rachis of the wheat spike and observed a 1 : 1 relation between spikelet number and the number of central vascular bundles at the base of the rachis, suggesting that a single vascular bundle is assigned to each spikelet (Whingwiri *et al.* 1981). The authors further observed branching of the main vascular bundle in the rachis and suggested that the vascular bundle connection between the rachis and the floret occurs after the initiation of the terminal spikelet (Whingwiri *et al.* 1981; Whingwiri and Stern 1982). Further, these researchers have also observed the decline in the number and size of vascular bundles acropetally, i.e. from the base to the apex of the rachis. However, apart from the presence of more vascular bundles in the more basal sections of the spike, it is not yet clear whether basal spikelets do benefit sufficiently from the seemingly available assimilate supply route.

Kirby also studied the vascular system of the barley spike and observed that the differentiation of phloem and xylem elements first starts in the lower mid part of the spike and then proceeds in a proximo-distal manner along the spike (Kirby and Rymer 1974). This suggests that the vascular bundles of spikelets in central spike positions are established first compared with those in the apical and basal spikelets. Thus, despite the presence of more vascular bundles in the basal section of the spike, and the proximity of the basal spikelets to the main assimilate route in the peduncle, connection of the basal spikelets to vascular system might occur later as the connection of the spikelets to the vascular system proceeds from the centre to the base (Kirby and Rymer 1974). This might also explain the 1 : 1 correlation between spikelet number and central vascular bundles at the base of the wheat spike observed by Whingwiri *et al.* (1981).

Before the observations of Whingwiri *et al.* (1981), Hanif and Langer (1972) also suggested that florets closer to the rachis node, i.e. the basal three florets in the spikelets, were directly supplied by the principal vascular bundles of the rachilla, while the distal florets lack a direct connection to the vascular bundle (Hanif and Langer 1972). This suggests that the distal florets might be subject to some mechanical modifications in the vascular connectivity and assimilate translocation. Therefore, distal florets might not have an equal chance of accessing assimilates from the source.

In line with the fact that only those few florets close to the main vascular system of the rachis are fertile and set grains, Bremner and Rawson (1978) also suggested the physical distance of the distal floret from the rachis as a cause for abortion. By changing the dynamics of assimilate supply through de-tillering, Guo and Schnurbusch (2015) also found a significant delay in the degeneration of the floral primordia resulting in increased floret fertility. Hence, such a sequential pattern of floret abortion in a wheat spikelet (González-Navarro *et al.* 2015; Guo and Schnurbusch 2015) suggests certain regulation of assimilate loading to the florets.

Although Kirby and Rymer (1974) and Whingwiri *et al.* (1981) independently suggested branching of the main vascular bundles in barley as well as wheat spike rachis (Kirby and Rymer 1974; Whingwiri *et al.* 1981), it is not yet clear as to how the assimilates are allocated to each of the branch units, i.e. the spikelets and florets. Hence, knowledge of the development of the vascular tissues, especially the main conducting elements i.e. the sieve tube elements, and their

architectural configurations in wheat spikelet/floret is very essential. Nevertheless, our knowledge and even theoretical working models in this regard is very limiting in wheat.

Based on a literature review, two contrasting models – the ‘pipe’ (Shinozaki *et al.* 1964) and ‘aorta’ models (McCulloh *et al.* 2003) – were suggested as working models for the architectural configuration of the vascular network system in plants. The main differences between the aorta and the pipe model is that the aorta transport networks are composed of several branching tube networks; while the pipe model is composed of multiple parallel or diverging vessels or tubes (McCulloh *et al.* 2003; McCulloh and Sperry 2006; Seki *et al.* 2015). Hence, unlike the pipe model, bifurcation of the mother tube into daughter tubes is a typical characteristic of the aorta model (Murray 1926; McCulloh *et al.* 2003; Seki *et al.* 2015). Thus, the aorta model states that across branching generations, the sum of the conduit radii (r) cubed at every level (Σr^3) is proportional to the volume flow rate at any point (Murray 1926; McCulloh *et al.* 2004; McCulloh and Sperry 2005). This principle is also independent of the vessel length and branching pattern. Hence, Σr^3 is conserved at any cross-section in the system (McCulloh *et al.* 2004). In contrast, each tube in the pipe model is considered to be independent and thus, no exchange of solutions among the pipes i.e. neither of the pipes bifurcates nor tapers at any location (Shinozaki *et al.* 1964; West *et al.* 1997; West *et al.* 1999; Seki *et al.* 2015).

Even though the aorta model was initially proposed based on the mammalian system describing the functional relationship between vessel radii and the volumetric flow during the branching of vasculature of the mammalian circulatory system (Murray 1926; Sherman 1981), both models, i.e. the aorta and pipe models, were also tested in plants (West *et al.* 1999; McCulloh *et al.* 2003, 2004; McCulloh and Sperry 2005; Savage *et al.* 2010; Price *et al.* 2013) including the critics on the model (Kozłowski and Konarzewski 2004, 2005). So far, the transport of water in plants, i.e. in the xylem and the branching order in the leaf vein geometries, follow the aorta model or Murray’s law (McCulloh *et al.* 2003; Price *et al.* 2013). Different studies have also suggested that the optimal supply and resource delivery in the external branching network might have also coevolved with internal vascular network system (West *et al.* 1997; West *et al.* 1999; Savage *et al.* 2010).

Unlike the mammalian aorta model, where Σr^3 is conserved at any cross-section, diminishing of Σr^3 has been reported in a plant system to the decline in volume flow rate (McCulloh *et al.* 2004; McCulloh and Sperry 2005). Also, the law still proved to be true even under such diminishing Σr^3 , provided that the reduction is in a direct proportion to the decline in volume flow rate (McCulloh *et al.* 2004; McCulloh and Sperry 2005). Furthermore, Murray’s law takes into account the cost of building, maintaining and transporting fluid, as well as the maximisation of the hydraulic conductance of the blood vessels across the branching points that also fit to the xylem system of seed plants (McCulloh *et al.* 2003; McCulloh and Sperry 2006; Price *et al.* 2013). Recently, Seki *et al.* (2015) mathematically predicted that phloem sucrose transport in the rice panicle is also likely to obey the aorta model (Seki *et al.* 2015).

Regardless of its importance, little is known about how sieve tube elements are distributed in wheat spikelets and how a single

tube bifurcates at each junction of the branches. Therefore, developing the appropriate genetic material and the mechanistic working model are important to further study the phloem anatomy and structure in wheat spikelets. Previously, we developed FL-*bh¹-A1*-NILs, which are typically characterised by the formation of supernumerary secondary spikelets (SSt) sharing the same rachis node with the primary spikelets (PSt) (Wolde *et al.* 2019a). The unique spikelet arrangement in FL-*bh¹-A1*-NILs, i.e. PST and SSt, which are connected to a similar assimilate outlet (the rachis node) provides a compelling opportunity to further study source–sink relationship, spikelet fertility, and the dynamics of assimilate partitioning and dry matter accumulation between wheat spikelets. In this study, we have systematically manipulated the dynamics of assimilate supply through de-tillering and investigated spikelet fertility and assimilate partitioning and dry matter accumulation between the PST and SSt. Even though the PST and SSt share the same rachis node, the differential spikelet fertility index and dry matter accumulation between PST and SSt suggested a tighter regulation of assimilate supply to the individual spikelets in wheat. This possibly indicates that there might be an underlying mechanism controlling assimilates allocation and distribution between wheat spikelets, especially limiting distal florets from sufficiently accessing the assimilate route. We will further discuss our results based on the suggested modalities on the architectural configuration of the vascular network system in plants.

Materials and methods

We used cv. Floradur Near Isogenic Lines (FL-*bh¹-A1*-NILs) carrying alleles of the *branched head¹* (*bh¹-A1*) locus derived from tetraploid wheat mutant accession, TRI 19165 or ‘Miracle Wheat’ (Poursarebani *et al.* 2015; Wolde *et al.* 2019a). Floradur is a German commercial elite spring durum wheat (*Triticum durum* L.) variety with a standard spike. Five different plant families of FL-*bh¹-A1*-NILs (at BC2F3, BC3F2 and BC3F3 generations) were used in this study. Generally, besides carrying the primary standard spikelets (PSt), FL-*bh¹-A1*-NILs are characterised by carrying secondary spikelets (SSSt) sharing the same rachis node with that of the PST (Wolde *et al.* 2019a). First, the five homozygous BC3F2 family plants ($n = 25$) were evaluated for spike morphology under the field conditions at the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany (51°49′23″N, 11°17′13″E, altitude 112 m above sea level) during the 2015 growing season. Grains were first sown in 96 well trays under controlled long day conditions i.e. 16/8 h day/night and 19/17°C day/night temperatures for 15 days. Then, plants were vernalised for about four weeks at 4°C. After 1 week of hardening at 15/12°C day/night temperature, seedlings were transplanted in the field characterised by silty loam soil. The distance between plants and rows was ~10 and 20 cm respectively. All spikes from each plant were harvested and the PST and SSSt were processed separately from the remaining spikelets. Spikelet fertility (grain number per spikelet), grain weight (thousand kernel weight) and grain morphometric traits: grain length, grain width, and grain area were analysed using Marvin digital seed

analyser (GAT Sensoric GmbH). An additional experiment was set up in the greenhouse under controlled conditions based on the five plant families (BC3F3 generation). Seven grains per family were germinated on 96 well trays under controlled long day conditions: 16/8 h day/night and 19/17°C day/night temperatures for 15 days. After 15 days of seedling establishment, plants were vernalised for about four weeks at 4°C. After 1 week of hardening at 15/12°C day/night temperature, each seedling was transferred to a pot size 0.5 L filled with substrate 2 (Klasmann-Deilmann GmbH, 49744 Geeste), compost and sand on a proportion of 2 : 2 : 1 respectively. Each plant received 10 g of NPK fertiliser called Plantacote Depot 4M (Wilhelm Haug GmbH and Co.) 1 week after potting. Plants were grouped into two major groups in three biological replicates for each group. About 35 plants per replicate were used in each group. Each pot was randomly arranged within the group and each plant received all the standard treatments (light, water, fertilizer, disease and pest controls) uniformly. Tiller removal experiment was conducted to group 1 plants (DT plants) while those in group 2 were allowed to freely tiller (FT plants). Tillers were always checked and removed every three days until maturity. Traits such as tiller number (TN), heading date (HD), plant height (PH), peduncle length (PdL), spike length (SL), spike dry weight at harvest (SDW_H), node number per spike (NPS), node density (ND), number of secondary spikelets per spike (SSSt), total spikelet per spike (sum of PST and SSSt), grain number per spike (GNS), grain number per spikelet (GPS), and thousand kernel weight (TKW) from the main culm were analysed. All grain related traits from PST and SSSt sharing the same rachis node were analysed separately to investigate the dynamics of spikelet fertility and dry matter accumulation in the grains. Mean phenotypic data from the three replications were subjected to unpaired two-tailed Student’s *t*-test.

Results

Spike architecture of the FL-bh¹-A1-NILs (BC3F3)

Canonical spikelets in wheat are distichously arranged on the main spike axis (rachis) and each rachis node usually bears only a single spikelet. Due to the introgressed *branched head¹* or *bh¹-A1* allele from ‘miracle wheat’, FL-*bh¹-A1*-NILs carry SSSt that share the same rachis node with the PST in a ventral-to-ventral orientation, more or less facing each other (Fig. 1b–d). The ‘paired spikelet’ phenotype, however, seen in many other forms, for example in (Boden *et al.* 2015; Dixon *et al.* 2018) is distinct in the way that spikelets, i.e. PST and SSSt, share one rachis node in a clearly dorsal-to-ventral fashion, rather resembling a piggy-back situation. Although there are clear anatomical differences among different non-canonical spikelet types, collectively, all of these ‘additional spikelets’ are often subsumed as ‘supernumerary spikelets’.

Here, it is important to note that spike-branching in ‘miracle wheat’ or TRI 19165, the donor of the *bh¹-A1* allele on chromosome 2A, is a true type or genuine spike-branching accession, whereupon ‘mini spike-like branches’ appear from the distichously arranged rachis nodes by replacing the spikelets (Poursarebani *et al.* 2015; Wolde *et al.* 2019b). However, FL-*bh¹-A1*-NILs predominantly show supernumerary spikelets without genuine spike-branching (Wolde *et al.* 2019a). This suggests the

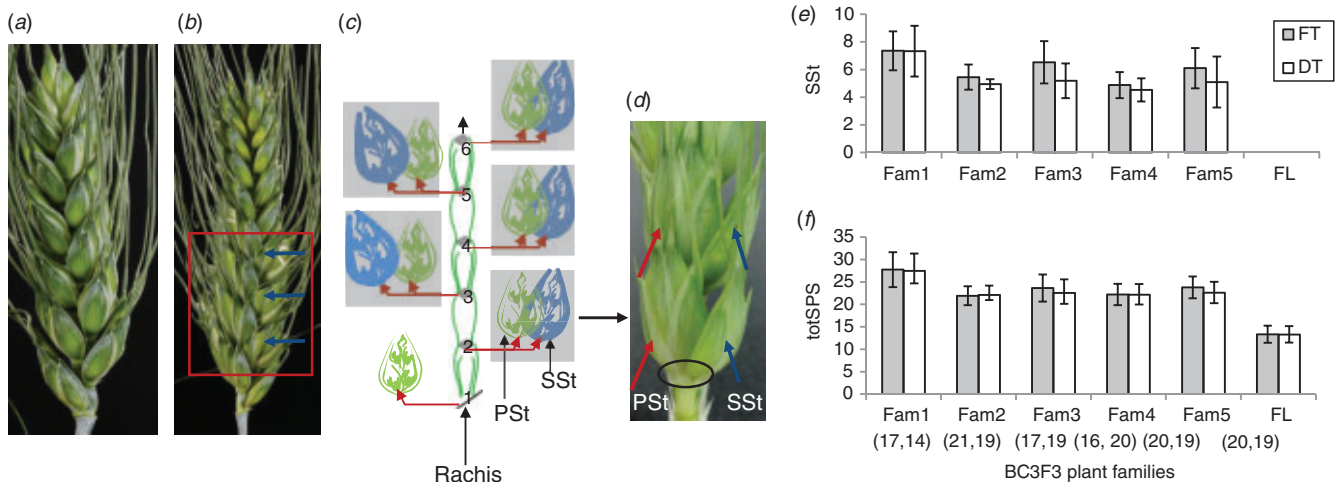


Fig. 1. Spike architecture of FL-*bh*¹-*A1*-NILs (BC3F3). (a) Floradur, (b) FL-*bh*¹-*A1*-NILs. The box shows the section of the spike where PSt and SSSt frequently appear. Arrow indicates the SSSt. (c) Schematic showing PSt (green) and SSSt (blue) of FL-*bh*¹-*A1*-NILs shown in (b) in boxed area. (d) Closer view of the PSt and SSSt on the rachis of the spike. Only a small section of the spike is shown. The circle indicates the rachis node that is shared by the PSt and SSSt. PSt and SSSt are arranged in a ventral-to-ventral orientation. (e) SSSt per spike. (f) Total spikelet per spike from five different FL-*bh*¹-*A1*-NILs (BC3F3) plant families. The bar indicates phenotypic mean $\pm$ s.d. The number in the parenthesis indicates sample size in the FT and DT plants respectively. Abbreviations: Fam, family; FL, Floradur (recurrent parent); FT, free-tillering; DT, de-tillering.

presence of other spike-branching modifiers suppressing genuine spike-branching in the recurrent parent, Floradur, background.

The response of spike development after source-sink manipulation (de-tillering)

Apart from the increase in SL of DT plants, no change in spikelet number between FT and DT plants was observed (Fig. 1e, f; Table S1, available as Supplementary Material to this paper). Hence, ND (number of nodes per unit length of the spike) in DT plants was lower than FT plants, indicating that DT plants somehow showed an elongated rachis internode. This might be one of the reasons for higher chaff weight in the DT plants (Table S1). Despite the increased SDW_H of the DT plants (Fig. 2b), no significant difference for spike harvest index (SHI) between FT and DT plants was found (Table S1), suggesting an inefficient conversion of biomass to the final grain weight. However, compared with the FT plants, the DT plants showed increased spikelet fertility (GPS) both in the PSt and SSSt which led to increased grain number per spike (Fig. 2d). Improved spikelet fertility after de-tillering (Fig. 2c) suggested source linked problems which might be linked with the translocation and/or distribution of assimilates.

Source-sink manipulation and the dynamics of spikelet fertility in the PSt and SSSt

Before we set up source-sink manipulation under greenhouse conditions, we studied spikelet fertility in the PSt and SSSt from plants grown under field conditions. The study was conducted using homozygous FL-*bh*¹-*A1*-NILs at BC2F3 and BC3F2 generations comprising of 28 and 25 individual plants respectively. Spikelet fertility between the PSt and SSSt was measured in terms of GPS from each of these

spikelets. Results showed that spikelet fertility and dry matter accumulation in the SSSt was always significantly lower than in the PSt (Fig. 3a, b). Even though PSt and SSSt share the same rachis node (i.e. potentially connected to similar assimilate sources) (Fig. 1b–d), the differential spikelet fertility and dry matter accumulation suggest a distinctive, yet unknown factor controlling assimilate partitioning and dry matter accumulation in wheat spikelets.

Although spikelet primordia in spike-branching or ‘miracle wheat’ mutants appear indeterminately (Shitsukawa *et al.* 2009; Poursarebani *et al.* 2015), SSSt might also be developmentally delayed compared with the PSt. Although we have not studied the developmental differences that might exist between the PSt and SSSt, we reasoned that the observed difference in spikelet fertility and dry matter accumulation between PSt and SSSt might also be due to the differences in ‘sink strength’ and hence, competition effects. Therefore, we conducted a greenhouse experiment to study the dynamics of assimilate partitioning and dry matter accumulation in the PSt and SSSt by diverting more resources to the main culm through de-tillering (see ‘Materials and methods’ for details).

Results showed a concomitant improvement of spikelet fertility both in the PSt and SSSt even after de-tillering (Fig. 4). We noted that unlike the SSSt in FT plants, SSSt in the DT plants showed up to 65% improvements in spikelet fertility, suggesting that the lowered spikelet fertility of the SSSt in the FT plants was due, partly, to assimilate supply and/or competition effect. However, the SSSt always tends to have lowered spikelet fertility compared with the corresponding PSt even after de-tillering, clearly indicating that more assimilates were translocated to the PSt compared with SSSt in all conditions. Nevertheless, the concomitant improvement of assimilate partitioning to the PSt and SSSt in the DT plants further suggested the tighter regulation of assimilate supply to the

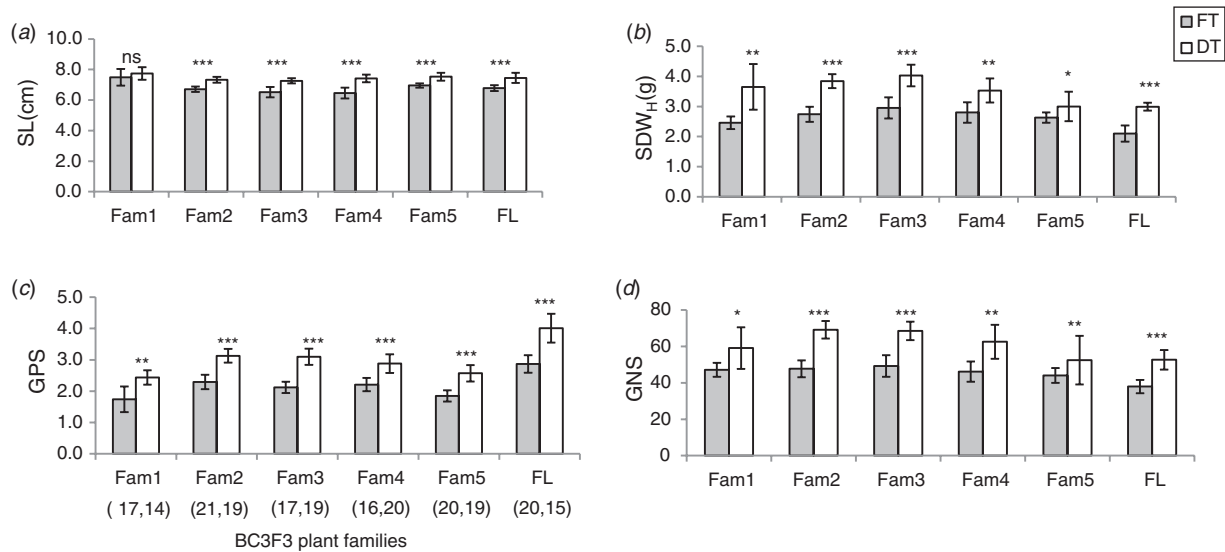


Fig. 2. Response of spike traits after source-sink manipulation (de-tillering). (a) Spike length (SL), (b) spike dry weight at harvest (SDW_H), (c) grains per spikelet (GPS), and (d) grain number per spike (GNS) from five different FL-*bh-A1'*-NILs (BC3F3) plant families. The bar indicates phenotypic mean $\pm$ s.d. *P*-values (0.05, 0.01 and 0.001) were determined by two-tailed, two-sample *t*-test. The number in the parenthesis indicates sample size in the FT and DT plants respectively. Abbreviations: Fam, family; FL, Floradur; FT, free-tillering; DT, de-tillering.

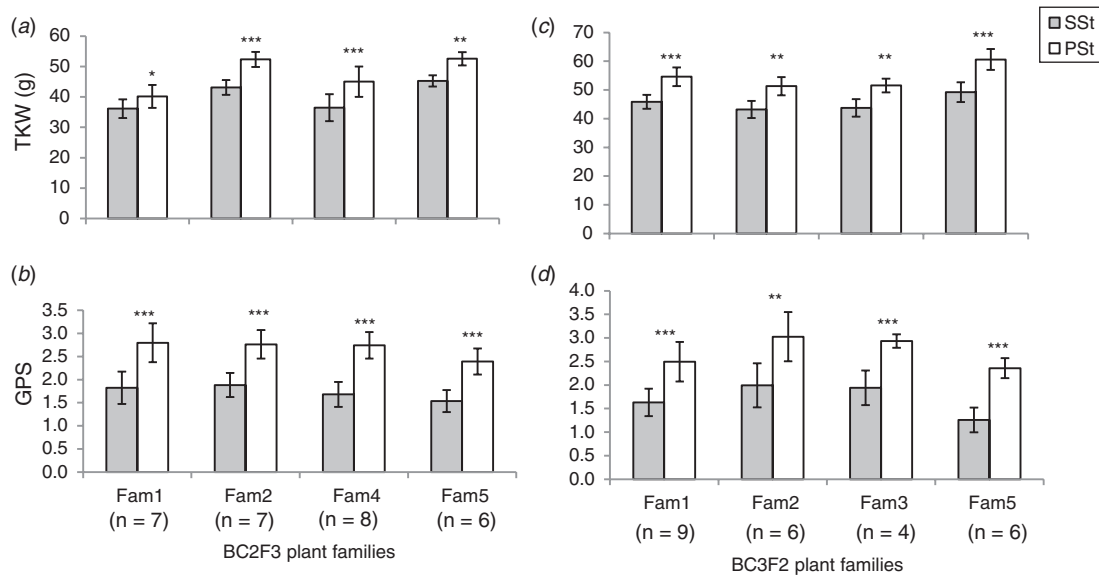


Fig. 3. Comparison of spikelet fertility and dry matter accumulation between PSt and SSt at field conditions. (a) Thousand kernel weight (TKW) and (b) grain number per spikelet (GPS) from four FL-*bh-A1*-NILs (BC2F3) plant families comprising 28 individual homozygous plants. (c) TKW and (d) GPS from four plant families of FL-*bh'-A1*-NILs (BC3F2) comprising 25 individual homozygous plants. The bar indicates phenotypic mean $\pm$ s.d. *P*-values (0.05, 0.01 and 0.001) were determined by two-tailed, two-sample *t*-test. The number in the parenthesis indicates sample size. Abbreviations: Fam, family.

individual spikelets in wheat. Thus, to better understand the regulation of assimilate partitioning to the individual wheat spikelets, especially from the perspectives of the conducting sieve tube elements, it is very important to dissect the temporal and spatial mechanics of vascular bundle development

between the PSt and SSt. As ovary (carpel) size in wheat was also shown to be one of the factors in floret and grain survival in wheat (Xie *et al.* 2015; Guo *et al.* 2016), it would also be worthwhile to conduct further studies on the floral development in the PSt and SSt.

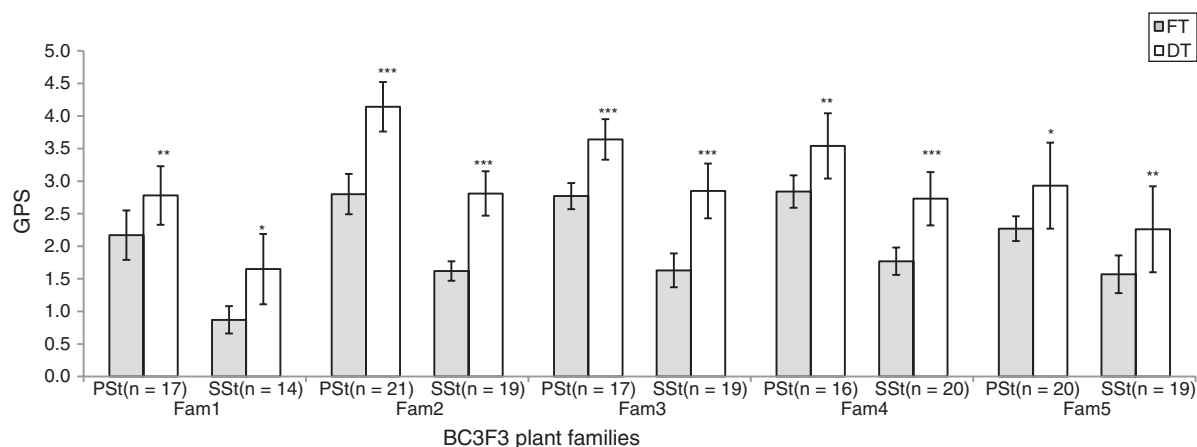


Fig. 4. Spikelet fertility between PSt and SSt after de-tillering. The bar indicates phenotypic mean $\pm$ s.d. *P*-values (0.05, 0.01 and 0.001) were determined by two-tailed, two-sample *t*-test. The number in the parenthesis indicates sample size in the FT and DT plants respectively. Abbreviations: Fam, family; FT, free-tillering; DT, de-tillered.

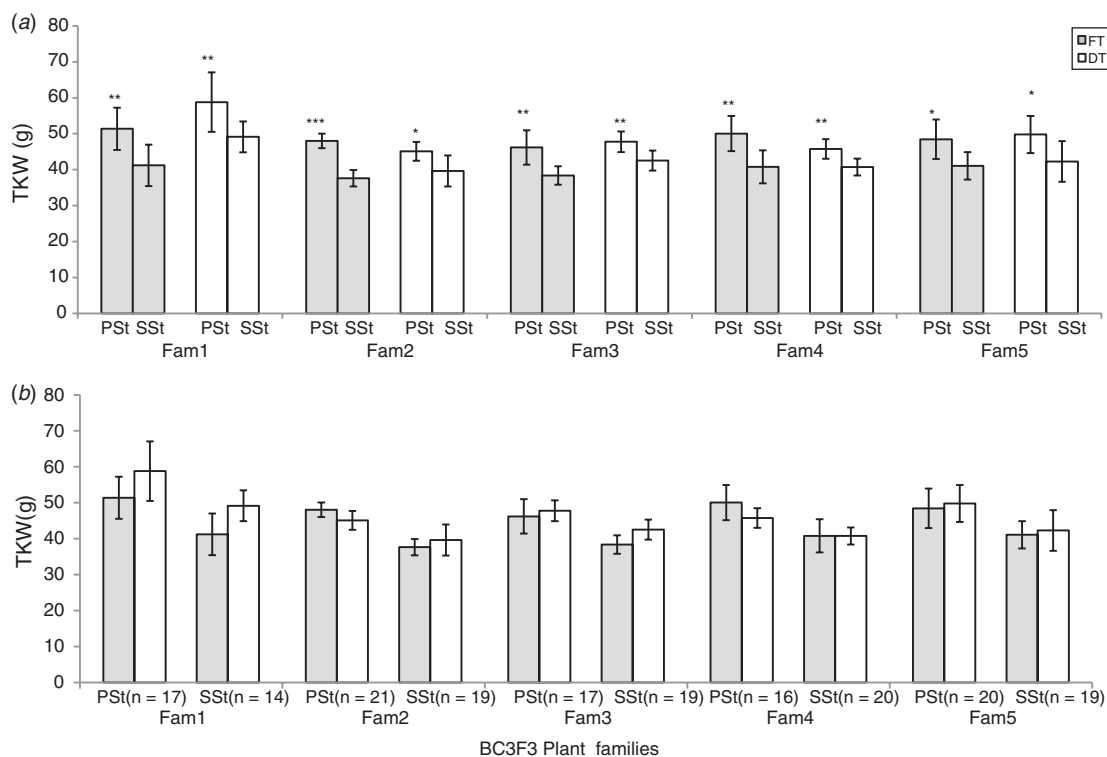


Fig. 5. Dry matter accumulation (thousand kernel weight, TKW) in the PSt and SSt. (a) Dry matter accumulation was significantly different between the PSt and SSt. (b) No effect of de-tillering on TKW both on the PSt and SSt. This might be due to the negative correlation between grain number and TKW. The bar indicates phenotypic mean $\pm$ s.d. *P*-values (0.05, 0.01 and 0.001) were determined by two-tailed, two-sample *t*-test. The number in the parenthesis indicates sample size. Abbreviations: Fam, family; FT, free-tillering; DT, de-tillered.

Source-sink manipulation and the dynamics of dry matter accumulation in the PSt and SSt

Thousand kernel weight (TKW) and the grain morphometric traits, i.e. grain length, grain width and grain area, were used to analyse grain development and dry matter accumulation in

the PSt and SSt (Fig. 5). De-tillering did not significantly affect the TKW of the grains in the PSt or SSt (Fig. 5b). Nevertheless, the differences in TKW of grains between the PSt and SSt both in the FT and DT plants was significant (Fig. 5a). This suggests that besides the variation in spikelet fertility between the PSt and SSt (Fig. 4), there was a clear

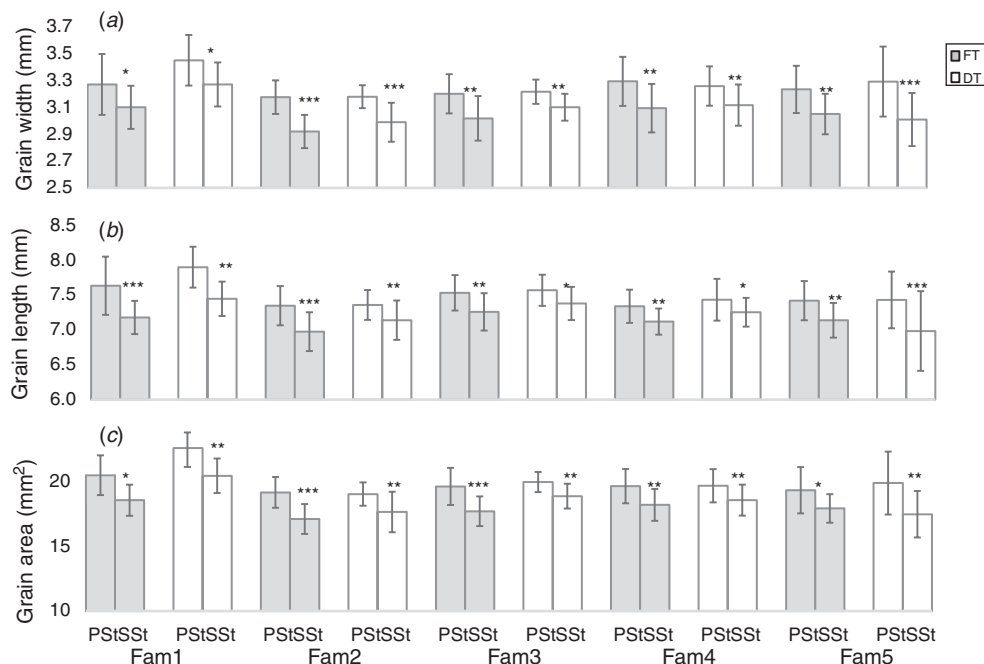


Fig. 6. Grain morphometric traits between PSt and SSt. (a) Grain width, (b) grain length, and (c) grain area. The bar indicates phenotypic mean $\pm$ s.d. *P*-values (0.05, 0.01 and 0.001) were determined by two-tailed, two-sample *t*-test. Abbreviations: Fam, family; FT, free-tillering; DT, de-tillering.

variation in dry matter accumulation and partitioning in the PSt and SSt even though both are connected to the same rachis node. We further analysed grain morphometric traits in the PSt and SSt (Fig. 6). Results showed that the grains in the PSt depicted enhanced grain length, grain width, and grain area compared with the grains in the SSt. These results show that besides the variation in spikelet fertility, there is also variation in grain development and dry matter accumulation in the PSt and SSt.

Discussion

FL-bh¹-A1-NILs represent novel spike morphology for use in wheat breeding

The genetic gains in wheat yield due to the introduction of semi-dwarf wheat varieties are associated with the partitioning of more photosynthate to the developing spikelets (Siddique *et al.* 1989; Miralles *et al.* 1998; Pearce *et al.* 2011; Thomas 2017). Nevertheless, the change in spikelet number per spike has been minimal (Royo *et al.* 2007; Álvaro *et al.* 2008; Philipp *et al.* 2018). As the modern wheat cultivars are also gradually approaching the theoretical upper limit for harvest index (Austin *et al.* 1980; Gaju *et al.* 2014), wheat breeding for new spike types is becoming imminent. Previously, the ‘ideal wheat plant ideotype’ characterised as mono-culm, lodging resistant with larger spike or sink size was proposed by Donald (1968). Such concept of a ‘communal wheat ideotype’, which defines a model of the idealised theoretical crop plant architecture under the assumption of minimised intra-specific growth competition, has never been tested experimentally in wheat. In the present study we phenocopied the mono-culm characteristics through de-tillering (DT plants) and a genetically controlled spike or

sink size, i.e. *FL-bh¹-A1-NILs*, which carry more spikelets per spike (Fig. 1*b, e*) (Wolde *et al.* 2019*a*).

Although *FL-bh¹-A1-NILs* are free tillering, and noting the fact that fertile tillers contribute to yield in wheat, our results clearly highlighted the benefit of having a single (mono) culm for improved spikelet fertility (Fig. 4). Previously, studies have also linked tiller abortion with the onset of stem elongation and anthesis suggesting that tiller death (abortion) is due, partly to resource competition (Mohamed and Marshall 1979; Kemp and Whingwiri 1980; Fraser *et al.* 1982; Davidson and Chevalier 1990; Berry *et al.* 2003; Xie *et al.* 2016). This suggests a benefit of resource diversion to the main culm for improved spikelet fertility that is otherwise spent on tiller development and maintenance, the fate of which is usually uncertain. We note that reports on the benefit of tiller inhibition, *tin*, genes and their positive effects in improving the harvest index have also started to emerge (Duggan *et al.* 2005; Kebrom *et al.* 2012; Gaju *et al.* 2014; Hendriks *et al.* 2016). For example, Hendriks *et al.* (2016) indicated the influence of the *tin* gene on root–shoot carbon partitioning. Even though the effect of introgressing the tiller inhibition gene into *FL-bh¹-A1-NIL* is to be seen in the future, the DT plants suggest the benefit of having mono-culm ideotype for resource maximisation in wheat for improving the harvest index, and possibly the application of mono-culm wheat ideotype in wheat breeding by combining *bh¹-A1* and *tin* genes. Our data based on *FL-bh¹-A1-NILs* already suggested a breeding opportunity by moderately modifying spikelet number without significantly compromising the grain weight (Wolde *et al.* 2019*a*). We note that *FL-bh¹-A1-NILs* are also more lodging resistant due to the presence of the *Rht-B1* semi-dwarfing allele paving ways towards the creation of a wheat plant ideotype as suggested by Donald (1968).

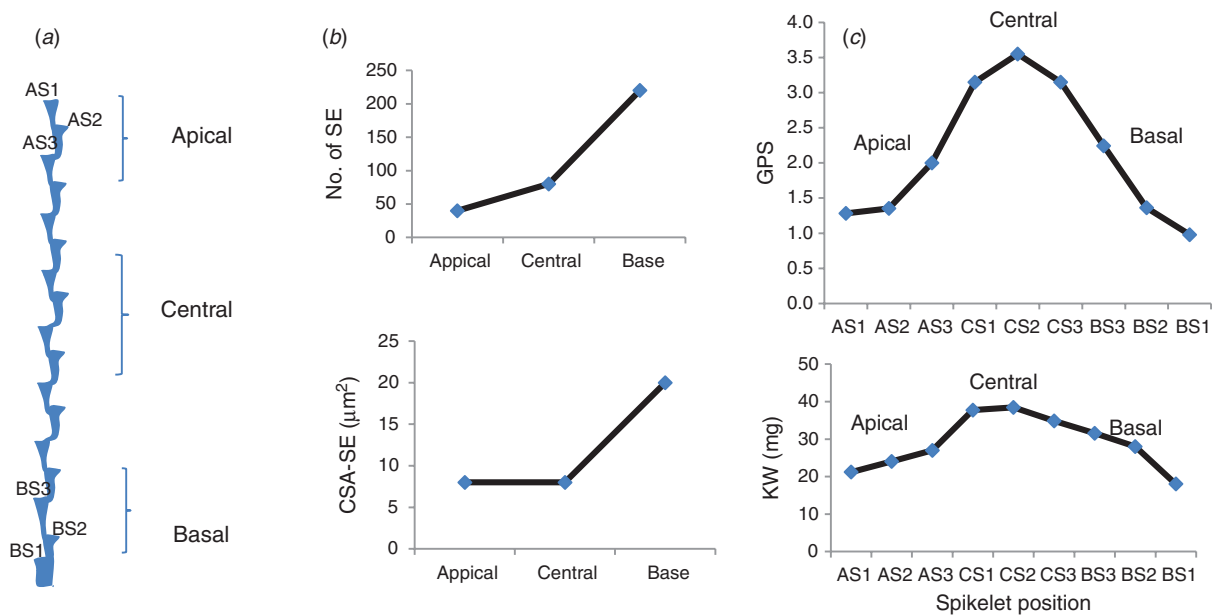


Fig. 7. Inferred distribution of spikelet fertility, dry matter accumulation and the number and size of sieve tube elements along a wheat spike. (a) Schematic of rachis of the wheat spike. (b) Number (top) and cross-sectional surface area (CSA) of the sieve tube elements (bottom) from three different positions of the rachis i.e. apical, central and basal. The graph was generated based on numerical data taken from fig. 6 in Whingwiri *et al.* (1981). (c) The inferred differential spikelet fertility (top) and dry matter accumulation (bottom) along the wheat spike. Data were combined from Guo *et al.* (2015), Li *et al.* (2016), and Whingwiri *et al.* (1981).

FL-bh¹-A1-NILs are suitable genetic materials to understand the dynamics of resource allocation in wheat spikelets

The wheat spike shows a differential spikelet fertility and dry matter accumulation along with the spike. For example, spikelets in the centre of the spike are more advanced, and hence possess higher grain number per spikelet than those spikelets at the apex and base (Bonnett 1936; Whingwiri *et al.* 1981; Whingwiri and Stern 1982; González-Navarro *et al.* 2015; Guo *et al.* 2015; Li *et al.* 2016; Philipp *et al.* 2018). This indicates that central spikelets have an advanced sink activity compared with those spikelets at a basal and apical section of the spike. Therefore, it is likely that the increased spikelet fertility of central spikelets is due, partly, to a better sink activity (Fig. 7b).

Often, SSt in *FL-bh¹-A1-NILs* appears in the bottom half of the spike (Fig. 1b). Although the time of initiation of the SSt is important in this regard, their unique structural arrangement, i.e. sharing same rachis node on the spike, is also quite important to elucidate on how resources are partitioned between wheat spikelets. Therefore, besides the differential spikelet fertility and dry matter accumulation along the spike, *FL-bh¹-A1-NILs* also have typical spikelet arrangement, i.e. PSt and SSt, showing a differential spikelet fertility (Fig. 4) and dry matter accumulation (Fig. 5), which suggests a tight regulation of resource allocation and dry matter accumulation among the wheat spikelets.

Besides sink activity, the sink size is also one of the most yield-limiting factors in wheat (Borrás *et al.* 2004; Miralles and Slafer 2007; Reynolds *et al.* 2007; Foulkes *et al.* 2011). Because of the increased spikelet number per spike followed by their unique arrangement along the spike, *FL-bh¹-A1-NILs* are important for the genetic and physiological studies of the source–sink relationship in wheat to better understand the

mechanism by which resources are partitioned among wheat spikelets to design future wheat spike ideotypes.

The discovery of the *Grain Number Increase 1 (GN1)* alleles also indicated that wheat plants can actively shutdown their yield potential by specifically aborting the most apical florets through apical rachilla degeneration (Sakuma *et al.* 2019). A disintegrated rachilla most likely works as an obstruction or constriction, depriving all more apically located florets and/or grains from any nutrients by simply starving them to death. This sort of ‘self-restriction’ is perhaps a useful evolutionary limitation in natural habitats, but is unwanted in attempts to maximise agricultural productivity. Therefore, besides the efforts to understand the genetic and physiological cause of floral abortion in wheat, a deeper understanding of the structural anatomy and allocation of resources in wheat spikelets and/or florets is essential.

Grain development and dry matter accumulation in FL-bh¹-A1-NILs

In addition to grain number, i.e. spikelet fertility, grain weight is also a major component for the determination of grain yield in wheat (Feng *et al.* 2018). Potentially, more grains can be set in each wheat spikelet, including in *FL-bh¹-A1-NILs*, but the relative contribution of each of these grains to TKW, and hence to yield, is variable due to uneven grain development and dry matter accumulation in the spikelet. For example, the relative contribution of distal grains (those that are farther from the base of the spikelet) to TKW is less than with those proximal to the rachis, i.e. at the base of the spikelet (Feng *et al.* 2018). *FL-bh¹-A1-NILs* show not only uneven grain development and dry matter accumulation within spikelets, but also uneven grain

development and dry matter accumulation between spikelets sharing same rachis node on the spike, i.e. PSt and SSt. Recently, it has been demonstrated that suppression of distal florets increases the weight of proximal grains by altering assimilate distribution (Golan *et al.* 2019), most likely as an effect of rachilla degeneration. Millet (1986) also reported a positive correlation between floret cavity size and the weight of the matching grain in wheat (Millet 1986). In this regard, differences in floral development between PSt and SSt, if any, might also partly contribute to the differences in grain weight between PSt and SSt. It has also been shown that carpel size, grain filling rate, and morphology are also determinant factors of grain weight in wheat (Xie *et al.* 2015).

TKW is also affected by grain shape and size (Su *et al.* 2018). These characteristics are determined by the temporal and spatial regulation of cell division, cell expansion, and the duration and rate of grain filling (Wheeler *et al.* 1996; Drea *et al.* 2005; Pielot *et al.* 2015; Li and Li 2016), which are dependent on the availability and acquisition of essential resources (Weber *et al.* 1998; Yang *et al.* 2004; Lopes *et al.* 2006; Egle *et al.* 2015; Weichert *et al.* 2017). We note that grain development in wheat spikelets follows an acropetal order (Kirby 1974; Whingwiri and Stern 1982), showing a decrease in grain weight or dry matter accumulation acropetally within the spikelets (Rawson and Evans 1970; Kirby 1974; Whingwiri *et al.* 1981; Guo *et al.* 2015). Since assimilate supply into the developing grain regulates the number and growth of endosperm cells, thereby affecting the dry matter accumulation (Brocklehurst 1977; Thorne 1985; Morita *et al.* 2005; Li and Li 2016), the acropetal reduction of grain weight within each spikelet might well be linked with efficiency of assimilate supply to the distal grains along the axis of the spikelet, i.e. the rachilla. Consistent with this, Borrás *et al.* (2004) and Jenner *et al.* (1991) have reported the influence of assimilate availability on grain dry matter accumulation (Jenner *et al.* 1991; Borrás *et al.* 2004).

Grain weight is also under the influence of the rate and duration of grain filling (Duguid and Brûlé-Babel 1994; Wang *et al.* 2008; Baillot *et al.* 2018). Since the main component of the wheat grain is an endosperm, genes regulating starch synthesis are essential for appropriate grain filling (Martínez-Barajas *et al.* 2011). This suggests that incomplete grain filling is due, in part, to defects in starch synthesis. Sucrose is the source for starch synthesis, and is transported from the source (leaves) into sink organs such as grains, so understanding sucrose delivery from the source to the developing grains is essential for designing novel approaches for improving wheat yield. To this end, the unique spikelet arrangement in *FL-bh¹-Al-NILs* is important to better understand the role of phloem architecture and the dynamics of sucrose delivery between and within the wheat spikelets.

Inferring the vascular architecture of the wheat spikelet

The application of a branched internal network system that was originally discovered based on the mammalian circulatory system, i.e. aorta model (Murray 1926), has been discovered in the plant system (McCulloh *et al.* 2003; Price *et al.* 2013; Seki *et al.* 2015). In this model, the mother tube bifurcates into daughter tubes, where the radius of the mother tube cubed will equal the sum of the cubes of the daughter radii (Murray

1926; LaBarbera 1990; McCulloh *et al.* 2003; Seki *et al.* 2015). However, with increased branching, the reduction in tube size (West *et al.* 1997; Seki *et al.* 2015) attracted our attention, so we were tempted to hypothesise on its application in relation to the general trend of floral abortion in wheat. Although our knowledge on the architectural configuration of the vascular bundle in the wheat spikelet is still incomplete, the acropetal decrease in spikelet fertility and dry matter accumulation within the wheat spikelet might be linked with a proportionate decrease in tube radii of the conducting tube elements in the spikelet. The schematic depiction of our hypothesis of the aorta model in wheat spikelet is shown in Fig. 8. Theoretically, floret abortion in wheat can also be explained, at least partially, by assuming that branching of the main vascular bundle follows the aorta model. Since the aorta model is independent of the length of the tube but affects the radius of the tube (McCulloh and Sperry 2006), further branching of the sieve tube elements within the wheat spikelet might limit assimilate supply to the distal florets (Fig. 8V). This could, in turn, cease cell division, and result in the interruption of the normal floral development in the distal florets.

The alternative model – the pipe model – seems to be less likely in wheat spikes because the assumption of the pipe model is that each conducting (sieve tube) element neither bifurcates nor tapers at any site (Seki *et al.* 2015) (Fig. 8S). Thus, conducting sieve tube elements are assumed to connect each spikelet/floret to the source (i.e. leaf); which means that each spikelet/floret have their own pipe that connects them directly to the source. Because competition for assimilates in the pipe model is believed to be distance-dependent (Seki *et al.* 2015), and the fact that the PSt and SSt share the same rachis node (Fig. 1d), they should have benefited equally, i.e. should have a similar spikelet fertility and dry matter accumulation index. However, the SSt has lower spikelet fertility and dry matter accumulation than the PSt both in FT and DT plants (Fig. 4). This difference might be attributed to the differential assimilate partitioning to each of these spikelets which might be linked with the varying tube radii of the vascular architecture, especially the conducting sieve tube elements in the PSt and SSt. If this is true, it is more compelling to hypothesise and model the vascular architecture from the basis of the principle and assumptions of the aorta model.

The second reason why the pipe model is less likely for the wheat spikelet is the very nature of the most basal wheat spikelets, which are often less fertile or even sterile (Fraser 1950; Kirby 1974; Guo *et al.* 2015; Li *et al.* 2016; Philipp *et al.* 2018). These spikelets have more vascular bundles and are closer to the source (leaf) than those from the centre or apical part of the spike (Fig. 7b), so according to the pipe model they should have benefited more than those in the central and/or apical portion of the spike simply because they are closer to the source (leaf). However, this is generally not the case in wheat. This suggests that the pipe model is again a less likely model for the wheat spikelet. Therefore, based on (i) the diminishing characteristics of Σr^3 with further branching in the modified version of the aorta model in plants combined with the inferred trend of distal floret degeneration and the acropetal decrease of the dry matter accumulation in each spikelet; and (ii) the inferred differential spikelet fertility index along the wheat spike, as well as spikelets sharing the same rachis node, i.e. PSt and SSt, it is

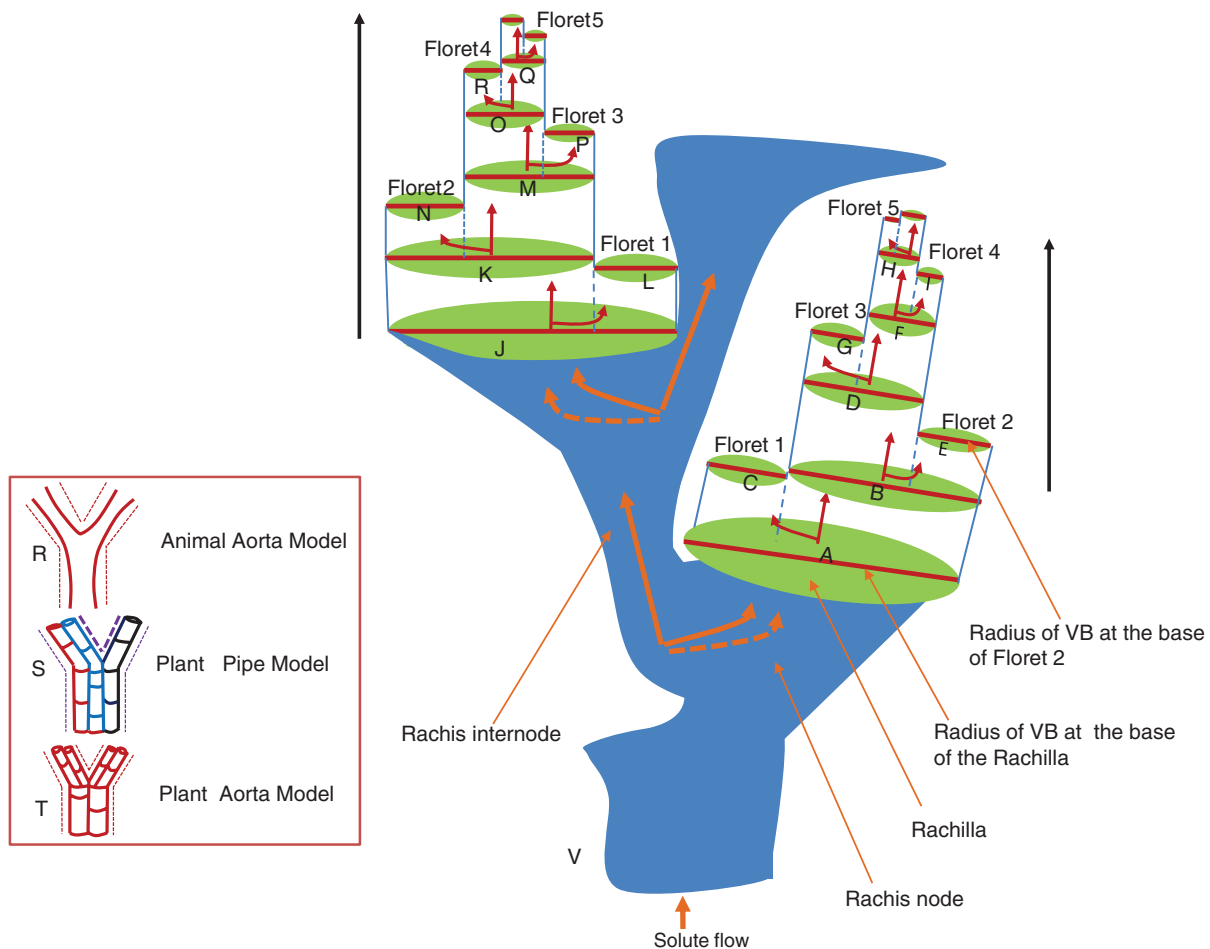


Fig. 8. Schematic depiction of the proposed aorta model in a wheat spike/spikelet. Inset figure (R, S, T) demonstrates aorta and pipe models. The figures were redrawn from McCulloh *et al.* (2003). (R) Aorta model in the mammalian system where a single continuous tube bifurcates into two smaller daughter tubes, (S) pipe model in plants where several non-branching conduits are running in parallel within each branch (colour coded), and (T) the aorta model in plants where the wide conduits at the base feed an increasing number of narrower conduits distally. (V) The proposed aorta model in a wheat spike/spikelet. Only the first two rachis nodes from the base of the rachis are shown. The black solid arrow indicates the direction of indeterminate floret primordia development in each spikelet axis (rachilla). At each rachis node; the vascular system is expected to bifurcate into two daughter tubes (solid orange arrows) in such a way that one of the daughter tube feeds the immediate spikelet while the other branch follows the rachis internode. Broken orange arrow indicates further branching of vascular system to the SSSt sharing the same rachis node with PSt (unbroken bend orange arrow). Since the wheat rachilla is indeterminate, further branching of the vascular system in each wheat spikelet is also expected to branch further to connect the florets. Hence, one branch feeds the immediate floret (i.e. floret 1) and the other branch follows rachilla internode which further bifurcates into two daughter tubes at the next node, where the daughter tubes feeds floret 2 and the other branch follows the rachilla internode to the next node and then further bifurcates following the same trend. If so, and by applying the aorta model during such scenario, where neither of the daughter tubes is thicker than its mother tube, we can assume different radii size that equal to the radius of the mother tube (Murray 1926; Seki *et al.* 2015). Thus, if we assume 'A' as the radius of the mother tube diverted from the rachis to the rachilla, then after bifurcation 'A' gives two daughter tubes of radii 'B' and 'C' where the sum of the radii of 'B' and 'C' equals the radius of 'A'. So, the vascular bundle (VB) in 'C' should feed floret 1. Tube 'B' further extends following the rachilla path until the next node where it is expected to bifurcate again into two daughter tubes of radii 'D' and 'E'. Then, 'E' feeds Floret 2 while 'D' further extends following the path of the rachilla until the next node following the same rule. Thus, the pattern of bifurcation of mother tube will follow the pattern: $A = B + C$; $B = D + E$; $D = F + G$; and $F = H + I$ and so forth.

likely that the aorta model is an imminent working model for assimilate transport network in wheat spikelet/floret.

Since the conducting tubes are not continuous in plants, i.e. divided into conduits that run in parallel (McCulloh *et al.* 2003), as shown in Fig. 8S and Fig. 8T, several of these conduits are approximated into one large tube per vascular bundle (Seki *et al.* 2015). Hence, after the split of the vascular bundle in the rachis/

rachilla, one branch goes to the immediate spikelet/floret and the other branches to rachis/rachilla internode (Fig. 8V).

Regardless of the fact that more vascular bundles are found at the base of the spike (Whingwiri *et al.* 1981) (Fig. 7b), it seems that the basal spikelets might not be directly connected to the main vascular bundle of the rachis. This could highlight that the branching of the main vascular bundle might start from the

centre of the spike and progresses in a proximo-distal fashion following the direction of spikelet differentiation (Bonnett 1936) where the reduction in the size of the main conducting elements might have contributed to the reduced spikelet/floret fertility in the distal spikelets/florets (Fig. 8).

Due to increased demand for carbohydrates by the developing spike, a sucrose feeding experiment through the flag leaf rescued more florets that otherwise aborted (Ghiglione *et al.* 2008). Consistent with this, enhanced spikelet fertility of SS_t after de-tillering (Fig. 4) also suggests that more florets are rescued from being aborted due, partly, to the diverted assimilate before it drops-off to the level that cannot sustain floret development anymore (Fig. 8). The sieve tube elements also transport amino acids and hormones (Ham and Lucas 2014). Thus, depletion of hormones and amino acids from the distal florets could also lead to similar effects.

Conclusion

A more detailed understanding of sink strength and source activity is fundamental for enhancing crop productivity. By developing FL-*bh¹-A1*-NILs, with increased sink size and spikelet arrangement, and by artificially manipulating the dynamics of the source–sink balance (through de-tillering), we observed differential spikelet fertility and dry matter accumulation patterns between sister spikelets, i.e. the PS_t and SS_t, sharing the rachis node, suggesting the action of important, but as yet unknown mechanisms controlling the process. Hence, a detailed understanding of the developmental and anatomical differences between the PS_t and SS_t will illustrate how resources are partitioned between similar sink organs, and lead towards a better understanding of floret and even grain abortion in wheat spikelets.

Conflicts of interest

The authors declare no conflicts of interest.

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