Physiological perspectives of reduced tillering and stunting in the tiller inhibition (tin) mutant of wheat

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Abstract. The number of tillers established in cereal crops far exceeds the number that end up being grain bearing at maturity. Improving the economy in tillering has been proposed to improve cereal yields in both favourable and unfavourable environments. The tiller inhibition mutant \textit{(tin)} is potentially useful for breeding varieties with a greater economy of tillering. However, its tendency to stunting under long day and low temperatures has limited its use. Recently, the inhibition of tillering in \textit{tin} has been linked to precocious development of solid basal internodes that compete for sucrose and possibly other resources with the growing tiller buds leading to their developmental arrest. Although the physiological basis of stunting in \textit{tin} is unknown, both inhibition of tillering and stunting begin during the transition from vegetative to reproductive phase indicating a common physiological basis for both. In this review, we provide overall perspectives for the physiological basis of tiller inhibition and stunting in \textit{tin} and suggest the direction of research in the future.

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Introduction

The \textit{tiller inhibition (tin)} mutant of wheat has most of the features of a high yielding ideal wheat plant for both favourable and dry environments as proposed by Donald (1968) and others. It has few tillers, enlarged or ‘gigas’ aboveground organs including thick leaves and stem, a large spike, more and larger grains per spikelet and a higher harvest index (Fig. 1\textit{a}, \textit{b}) (Atsmon and Jacobs 1977). The reduced tillering associated with \textit{tin} is potentially useful for breeding new wheat varieties as fewer sterile tillers are initiated presumably leading to an increased allocation of carbon to fewer but larger fertile tillers (i.e. ‘gigas’ features) that may be more suited to current planting practices in favourable environments (Donald 1968). This plant type may also be important for drier regions where reduced leaf area index may limit transpirational water loss up to anthesis so that more soil water is available for grain filling resulting in a higher harvest index and higher yield (Richards 1988; Duggan \textit{et al.} 2005\textit{a}, 2005\textit{b}). Some of these traits of \textit{tin} also resemble the domestication syndrome described for maize and other crops; compared with their wild relatives, domesticated crop species display increased apical dominance and overall enhanced growth of vegetative and reproductive structures (Doebley \textit{et al.} 2006). However, unlike the domestication of crops by ancient farmers through selection from a natural population thousands of years ago, \textit{tin} was identified in the 1970s by plant scientists in segregants from a cross between a North African landrace Mabruk and the Israeli cultivar Alpha (Atsmon and Jacobs 1977; Duggan \textit{et al.} 2002). The developmental mechanism attributed to \textit{tin} may not have been selected by ancient farmers because of an undesirable accompanying trait: its tendency to stunt in some genetic backgrounds under long day and low temperatures (Atsmon and Jacobs 1977; Atsmon \textit{et al.} 1986\textit{a}; Duggan \textit{et al.} 2002). Such opportunities to increase yield in wheat by deploying a recently identified gene that can radically alter plant morphology has been the reason for the physiological and genetic studies into the reduced tillering and stunting of \textit{tin} for the past three decades.

The expression of \textit{tin} varies according to the background genotype and to the environment in which it is grown, and can range from a plant that is stunted and produces no grain to unicumul (e.g. line 492), biculum, to almost free tillering. This range in expression can be generated by altering the conditions in which plants are grown but there is also a genetic component whereby levels of expression can be fixed. Most studies on \textit{tin} have been conducted on line 492, or derivatives from it, because of its robust ‘gigas’ phenotype. Line 492 originated from the North African landrace crossed to a commercial wheat from Israel. Line 492 and its derivatives have also been used extensively in crosses to cultivated varieties of wheat. The \textit{Tiller Inhibition (Tin)} gene has been mapped to the short arm of chromosome 1A and lines with the \textit{tin} gene, including the source line 492, have been referred as \textit{tin} mutants after the name of the locus (Richards 1988; Kebrom \textit{et al.} 2012). Recent physiological studies linked the reduced tillering of \textit{tin} to precocious development of solid basal internodes that competes for resources with the developing tillers leading to their developmental arrest (Fig. 1\textit{c}–\textit{e}) (Kebrom \textit{et al.} 2012). Dormancy induced by factors within the plant but outside the bud is referred to as para-dormancy or correlative inhibition. The study in \textit{tin} revealed a novel para-dormancy of axillary buds in wheat, which is different from the classic apical dominance of...
inhibition of axillary bud outgrowth by auxin from the shoot apex in eudicots. It is noteworthy that an overlap in the developmental stage, for the onset of tiller inhibition and stunting, during transition from vegetative to flowering phase, indicate a common physiological basis for both, with stunting a severe form of the tin phenotype (Atsmon et al. 1986a; Richards 1988; Kebrom et al. 2012). The objective of this review is to provide an overall physiological perspective for the reduced tillering and stunting in tin and suggest the direction of research in the future.

Agronomic advantages of reduced tillering

The reduction in sterile tillers and the production of large reproductive spikes, associated with the identification of a single locus for the tin gene, has made tin very attractive to breeders in their quest to increased yield. The morphology of tin plants matches Donald’s (1968) ideotype for high yield in favourable environments, but it also has advantages in dry environments. In dry years the reduced leaf area associated with tin may reduce crop transpiration and thereby crop water use up to anthesis so that more water is available for grain yield formation. A suite of other advantages associated with tin, particularly for dry environments have also been noted. For example, tin has been found to increase root growth (Duggan et al. 2005b; Richards et al. 2007), increase storage of carbohydrates that can later be remobilised to the growing grain (Richards et al. 2002) and increase kernel size in field plots (Duggan et al. 2005a; Mitchell et al. 2012). The latter has been found to result in less shrunken grains (screenings) which is desirable to avoid a penalty at grain receipt points (e.g. Mitchell et al. 2012). Numerous studies, especially in water-limited environments, have shown a yield advantage associated with reduced tillering in field studies (e.g. Donald 1979; Innes and Blackwell 1981; Islam and Sedgley 1981). Given the simple inheritance of tin it is straightforward to develop near-isogenic lines or populations that differ in the presence or absence of tin to evaluate in the field (Duggan et al. 2005a; Mitchell et al. 2012). Stunting in these lines in the environments where they have been grown is rare. This is largely due to the short days during vegetative growth but also partly because there has been some selection against extreme tiller inhibition and stunting in the selection of lines grown. Although showing the expected phenotype in these field studies, no consistent yield advantage of tin across all environments has yet been demonstrated. However, it is clear that tin lines in some genetic backgrounds can be either higher yielding or lower yielding than their corresponding non-tin lines, and that in some environments tin lines yield more. For the field studies contrasting tin with non-tin the gene has been introduced into free-tillering varieties previously selected for yield whereas there has been no selection for yield in the lines.
with tin. It is possible that selection for yield among tin progeny may result in higher yielding and better adapted lines. Another uncertainty with these field studies is that all lines are generally sown at the same planting density, resulting in a higher spike density in non-tin lines. Given that the tin phenotype is a radical departure in plant form from conventional cultivars then genotype specific agronomy may be required if the full potential of the tin gene is to be realised in commercial cultivars.

Expression of reduced tillering and stunting in tin depends on environmental, hormonal, and genetic factors

Most wheat varieties produce many tillers during the vegetative period, many of which are sterile. However, the tin mutant produces only about two to three tillers, which are fertile and ‘gigas’. The ‘gigas’ characteristics could be due to an indirect effect of reduced tillering because it can be simulated by de-tillering a near-isogenic wild type (Kebrom et al. 2012). Presumably the resources available for tiller initiation and their growth are redirected to the fewer tillers already expanding in tin. Stunted tin plants can vary in expression from an extreme form that is lethal, and where ears do not develop to a small fertile plant with a single spike. Stunting is observed early in development and plants are characterised by a compact growth habit due to reduced distance between the ligules of successively formed leaves (Duggan et al. 2002). Extreme expression of tiller inhibition and stunting in tin depends on daylength and temperature as well as other environmental conditions, some of which are still unknown (Fig. 2). For example, even when a fixed genotype is grown under seemingly identical conditions in a field plot there can be subtle differences within the field that induces a range in expression from stunting to ‘gigas’ biculm plants. Generally, short days stimulate tillering in tin, whereas tillering under long days was stimulated at temperatures above 21/16°C but not at lower temperatures (Atsmon et al. 1986b). Both tiller inhibition and stunting were observed only in long days when the day/night temperatures were lower than 21/16°C (Atsmon et al. 1986a, 1986b) indicating the same physiological basis for both. This is further supported by the finding that the time of onset of tiller inhibition and stunting was associated with the beginning of transition of the shoot apex from vegetative to reproductive stage (Inbal and Atsmon 1983; Duggan et al. 2002; Kebrom et al. 2012). Stunting was also more evident at higher irradiance and at night temperatures of 12°C or less but not at 19°C (Duggan et al. 2002). At 19°C day/night temperatures the number of tillers was higher possibly due to overall accelerated growth before reaching the reproductive developmental stage at which tiller inhibition usually begins. Mild stunting was also induced by high CO2 treatments as indicated by reduced distance between ligules of successive leaves (Duggan et al. 2002). The higher frequency of stunted plants, which were otherwise reduced in tillering and showed ‘gigas’ features, was associated with higher irradiance and elevated CO2 indicating assimilate supply to the apex before floral initiation may induce stunting.

The effect of plant hormones on stunting of tin was investigated by Inbal and Atsmon (1983). In this study, stunting in line 492 was induced by ethylene, abscisic acid (ABA) and the anti-auxin p-chloro-phenoxyisobutyric acid (PCIB) under non-inductive conditions of short day and low temperatures (SD/LT); auxin and the ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG) reduced stunting under inductive conditions of long day and low temperatures (LD/LT) (Fig. 2). Among the hormones, the effect of ethylene and AVG was more profound than the others in inducing or inhibiting stunting respectively. Ethylene induced severe stunting in line 492 but not in any other wheat varieties without tin, indicating the induction of stunting by ethylene is specific to tin, and may not be a general response of wheat plants (Christopher et al. 1985). No effect on stunting was observed when gibberellic acid (GA) or GA-inhibitor was applied. Cytokinin enhanced the development of the spike and had no effect on vegetative growth.

![Fig. 2. Stunting in tin line 492 is induced by environmental, developmental and hormonal factors. Stunting occurs under long day (LD) and low temperatures (LT) during transition from vegetative to flowering and early stages of reproductive development, but not in short days (SD). Under noninductive growing conditions stunting is induced by application of ethylene, ABA and PCIB whereas application of AVG and to a lesser extent auxin reduced the proportion of stunted plants under inductive conditions of long day and low temperatures. Stunting is also induced by high irradiance or elevated CO2.](image-url)
Low temperature and long days are important for both floral initiation and for extreme forms of tiller inhibition in lines with tin. However, there is little evidence that these are connected. For example, line 492 and the tin gene in the Banks background are both prone to stunting, yet sister lines to these also with tin and with the same flowering time do not stunt (Atsmon et al. 1986a; Duggan et al. 2002). Also, flowering time in these lines is largely insensitive to either vernalisation or photoperiod. Nevertheless, using a range of conditions to induce stunting Christopher et al. (1985) found stunting was more frequent in crosses between line 492 and earlier flowering Chinese Spring and its derivatives than with the later-flowering Chinese Spring and its derivatives. It is likely that tiller inhibition is expressed more in wheats that initiate floral primordia early and that when floral initiation is delayed, such as in Chinese Spring, then extreme tiller inhibition (and stunting) is less common.

The genetics of reduced tillering and stunting in tin

The onset of tiller inhibition and stunting occur concurrently, before the formation of double ridges on the apical meristem, and therefore, before the onset of any visible sign of reproductive development. This indicates a common physiological basis. Stunting is thought to be an extreme manifestation of reduced tillering that is induced by particular environmental conditions as it is found only in tin lines with an extreme expression of tiller inhibition. However, studies on the genetic basis of the tin phenotype do not lend support to a common physiological basis as stunting and reduced tillering have been mapped to different loci of the wheat genome even though line 492 was used as a common parent (Christopher et al. 1985; Richards 1988). Based on plant morphology in several F2 and backcross populations a single tin locus mapped to the distal region of the short arm of chromosome 1A (1AS) (Richards 1988). Molecular studies also corroborate the locus position on chromosome 1AS (Spielmeyer and Richards 2004). In these studies, plants were grown under long days and low temperatures and tiller number at maturity was counted. A single locus for stunting was also reported by Inbal and Atsmon (1983) in F2 and BC1 F2 populations grown under long day and low temperatures and by Christopher et al. (1985) in crosses of line 492 to six other spring wheats when grown under long days and low temperatures together with an ethylene releasing compound which induces stunting. However, in crosses between line 492 and Chinese Spring (which flowers much later than the other parent lines), two major genes for stunting were identified. Using a Chinese Spring monosomic series these genes were located on chromosome 4B and 5B with modifiers on chromosomes 2A, 2B, 3A and 3D (Christopher et al. 1985). The identification of more than two genes is not unexpected because line 492 was derived from a cross between two normal lines that did not stunt (Inbal and Atsmon 1983). Reasons for the discrepancies between the two studies are unclear. The main discrepancy occurs when Chinese Spring and its derivatives were used in crosses with a number of possible reasons: first, that Chinese Spring flowered 45 days after line 492 and this has influenced genetic control of tiller formation. It is notable that major loci regulating flowering time co-locate to several of the chromosomes identified by Christopher et al. (1985). Second, an ethylene compound was used to induce stunting in one study but not the other and third, the phenotype scored was different. Whether the action of the reduced tillering locus on 1AS is independent of the stunting genes needs further investigation. Furthermore, a re-evaluation of the inheritance of stunting may be necessary as recent advances in genomics may provide additional information for the genetic bases of tin phenotype. A more systematic study of the development of stunting may reveal its molecular and physiological basis as has been done recently for the reduction in tillering (Kebrom et al. 2012).

Stunting, like phenotypes such as apical lethality or hybrid dwarfism, has been reported in crosses between some wheat varieties (Hermson 1967; Tomar et al. 2007). Furthermore, abnormally dwarf plants are commonly observed in mutagenised populations of hexaploid wheats (TH Kebrom, pers. obs.) possibly due to having one recessive allele for dwarfism and the second being mutated. However, stunting in tin appears to be different from apical lethality and hybrid dwarfism (Inbal and Atsmon 1983; Christopher et al. 1985). The genetic basis of stunting and its similarity and differences to the other forms of dwarfism in wheat should be re-examined.

Physiological studies revealed a novel para-dormancy mechanism that inhibits tillering in tin

The wheat plant produces many primary and higher order tillers during the vegetative phase. Following transition to reproductive phase, tiller formation ceases and tiller senescence begins and coincides with the beginning of stem elongation (Hay 1986). Most wheat varieties cease tiller formation when the apex of the main shoot reaches terminal spikelet stage and internodes start to elongate (McMaster 1997). Unlike leaves, internodes are strong sink and storage organs, therefore, strong competitors for photoassimilates (Kirby 1988; Schnyder 1993). Tiller senescence during the reproductive stage could be due to diversion of resources to stem or internode elongation. In tin, internodes start to elongate before the terminal spikelet stage and it is associated with the early onset of inhibition of tiller bud outgrowth and reduced tillering (Kebrom et al. 2012). Furthermore, the level of sucrose in the buds of tin that stopped growing is lower. The mechanism of tiller inhibition identified in tin is a novel type of para-dormancy different from the classic apical dominance in eudicots regulated by auxin derived from the shoot tip (Kebrom et al. 2012).

Tillering or shoot branching has been studied for more than eight decades mainly in eudicots using physiological and genetic methods (reviewed by Beveridge et al. 2009; Domagalska and Leyser 2011). The development of a branch is a two-step process that involves initiation of meristems in the axil of a leaf to form a bud followed by bud outgrowth. Both initiation of axillary meristem and bud outgrowth are regulated by a complex interactions of endogenous and environmental factors and genetic mechanisms. Although, earlier studies of decapitation and application of plant hormones established that auxin synthesised in the shoot apex inhibits bud outgrowth (Skoog and Thimann 1934), recent studies revealed auxin from the shoot apex represses bud outgrowth indirectly by inhibiting the biosynthesis of cytokinins that promotes bud outgrowth and/or stimulating the biosynthesis of strigolactones that inhibit bud outgrowth (Beveridge et al. 2009). Buds must export auxin to the
stem in order to grow and strigolactones inhibit bud outgrowth by limiting the sink strength of the stem for auxin (Reviewed by Domagalska and Leyser 2011). The inhibitory signals generated by auxin are integrated within the bud through changes in the expression of the *Teosinte branched1* (*Tb1*) like genes (Domagalska and Leyser 2011; Müller and Leyser 2011). The *Tb1* like genes are also involved in integrating signals generated by environmental factors such as shade that determine the dormancy versus outgrowth fates of axillary buds (Müller and Leyser 2011; Kebrom et al. 2013). In *tin*, the inhibition of bud outgrowth is associated with increased expression of the *Tb1* gene in axillary buds indicating integration of response to sucrose level also through *Tb1* (Kebrom et al. 2012). The regulation of branching by hormonal and environmental signals is largely conserved between monocots and eudicots, with some variations in the molecular mechanisms due to differences in the genetic make-up or developmental strategies among species (Kebrom et al. 2013). Although, the role of sucrose supply in dormancy and outgrowth of axillary buds has been largely ignored, the results from *tin* indicate that sucrose could be a major factor in shoot branching. It is possible that the inhibition of tillering in *tin* may involve additional factors other than sucrose. In *tin* tiller inhibition is associated with early internode elongation suggesting that the *tin* gene regulates the timing of development of internodes. Physiological and molecular regulation of internode development identified in other species may provide clues on the function of the *tin* gene in wheat.

**Regulation of internode development**

The vegetative part of a plant is formed by the repetitive production of developmental units known as phytomers (McMaster 2005). A phytomer consists of a leaf, a node, an internode and axillary bud, all generated by the activities of the vegetative shoot apical meristem (SAM). In wheat, the phytomers formed during the vegetative phase do not form internodes. Following transition of the shoot apex from vegetative to flowering phase, the young phytomer just below the shoot apex develop short stem internode and successive phytomers continue to form longer internodes eventually raising the inflorescence from the soil surface up into the air. In some other grass species internodes develop during the vegetative phase indicating transition into flowering phase may not be a requirement for internode development (Evans 1964), whereas internodes in the model species *Arabidopsis*, maize and rice develop during the reproductive phase (Fournier and Andrieu 2000; Jacqmaud et al. 2003; Luo et al. 2006). Most wheat varieties initiate internodes during the reproductive phase after terminal spikelet formation (McMaster 1997) indicating a tight developmental regulatory mechanism for the timing of initiation of internode elongation. However, the molecular and hormonal regulatory mechanisms have been studied only in the model species of *Arabidopsis*, rice and maize.

The shoot apical meristem consists of the central, peripheral and rib zones (Ha et al. 2010). Leaf primordia are initiated from founder cells recruited from the peripheral zone and stem internodes develop from the peripheral and rib zones in the subapical region. The meristematic cells in the central zone go through cell division and refill the peripheral and rib zones that continuously lose cells for organ formation. Several studies indicate internode development during the vegetative phase might be repressed in part by preventing gibberellic acids (GAs) synthesised in leaves from diffusing into the subapical regions (Sakamoto et al. 2001; Bolduc and Hake 2009). Consistent with this, genes encoding GA deactivating enzymes, *GA2oxidases* (*GA2ox*s), are expressed around the subapical region of rice, maize and *Arabidopsis* plants during the vegetative phases (Sakamoto et al. 2001; Jasinski et al. 2005; Bolduc and Hake 2009). In maize, the expression of *GA2ox1* in the subapical region is regulated by the KNOTTED1-like (KNOX) homeobox transcription factor *kn1* (Bolduc and Hake 2009). The *OsGA2ox1* of rice is downregulated following transition to flowering phase and it is associated with the beginning of internode formation (Sakamoto et al. 2001). Preliminary studies also indicated a decline in the expression of the wheat (EST Accession number BF484693), orthologue of *GA2ox1* of maize and rice earlier in the subapical regions of the *tin* mutant compared with wild type (TH Kebrom, PM Chandler, W Spielmeyer, RA Richards, unpubl. data). A *GA2oxidase* has been implicated in the domestication of sunflower (Blackman et al. 2011), one of the crop species where its domestication was associated with enhanced apical dominance, thick stem and flower (Doebley et al. 2006). The KNOX transcription factors maintain meristematic identity of cells in the SAM and downregulated in organ founder cells (reviewed by Hamant and Pautot 2010; Hay and Tsiantis 2010). Their nuclear localisation and DNA binding activities are dependent on BELL-like (BELL) family of homeobox transcription factors (Bellouki et al. 2001; Cole et al. 2006). The Bell-type *ARABIDOPSIS THALIANA HOMEBOX GENE*1 (*ATH1*) also represses internode elongation during the vegetative phase and the mechanism appears to be independent of changes in the expression of *GA2oxidase* genes (Gomez-Mena and Sablowski 2008). The candidate for reduced tillering barley mutant, *Int1*, *JuBeL2* belongs to the BELL family of transcription factors (Dubert et al. 2010). These findings suggest the significance of regulation of internode development by the KNOX-BELL-GA2oxidase pathway in shoot branching or tillering (Kebrom et al. 2013). Several KNOX and BELL mutants including Rough sheath1 (Rs1) maize, *Oriza sativa* homeobox (osh15) of rice, and BREVIPEDICELLS (*bp*) and PENNYWISE (*pny*) of *Arabidopsis* are shorter indicating a more specific role for these genes in internode development (Becraft and Freehill 1994; Sato et al. 1999; Venglat et al. 2002; Smith and Hake 2003). The Rs1, *OSH15* and *BP* are orthologs.

In addition, internode elongation is also stimulated in response to flooding and high planting density or shade signals, and involves changes in the metabolism and signalling of the major plant hormones including auxin, cytokinin, ethylene, ABA and GA (Stamm and Kumar 2010; Bailey-Serres et al. 2012). Response to flooding in rice could be either internode elongation or stunting and both responses are mediated by ethylene (Nagai et al. 2010). In tobacco, application of lower doses of ethylene enhanced growth while a higher level inhibited growth indicating the positive and negative role of ethylene in plant growth (Pierik et al. 2006). The plant hormones auxin, ethylene and ABA are implicated in stunting...
in tin (Inbal and Atsmon 1983). Analyses of the regulation of internode development by plant hormones and KNOX/BELL transcription factors might give clues to the physiological basis of stunting in tin.

**Internode elongation but not initiation is inhibited in stunted tin plants**

Long day and low night temperatures induce stunting in tin (Fig. 2). However, the physiological and molecular basis of stunting is still unknown. Wheat is a long day plant. Induction of stunting at long but not short day growing conditions is consistent with the sensitivity of tin to stunting during the specific developmental window of transition to flowering and early reproductive phase of development. Therefore, long days might be required to promote transition to flowering and internode development but may not induce stunting. Consistent with this, long day and higher temperatures do not induce stunting. Stunting is induced by low temperatures following transition to reproductive stage. Stunted plants display a compact growth habit – the internodes are short and spike development is retarded (Fig. 3). However, whether the compact growth habit and retarded spike development are associated or not with the failure of the internodes to properly develop is yet to be investigated.

Although the inhibition of tillering is associated with early and solid internode elongation, stunting appears to be associated with failure of the internodes to elongate once they are initiated. Indeed, internode number in stunted tin plants is not affected (Fig. 3) and sometimes stunted plants can form internodes faster than normal plants (Kebrom et al. 2012). The Rs1, bp and osh15 mutants are defective in internode elongation and are dwarf. However, the number of internodes in Rs1 was not affected (Becraft and Freeing 1994). We note that in the osh15 mutant only the lower internodes are affected but the length of the uppermost internode is similar to the wild type indicating that the OSH15 regulates the elongation of the basal internodes (Sato et al. 1999). In tin, only the basal internodes are unique in that they are solid indicating that tin is defective in regulating the development of these basal internodes. In addition, stunting in line 492 can be induced when the plants are grown under long day and low temperatures during the 2–4 weeks after planting (Atsmon et al. 1986a) and this period may span the developmental window for the initiation and development of the basal internodes. Stunted plants that escape and develop a fertile spike might have survived the sensitive developmental stages of basal internode development with minor damage that do not interfere with their subsequent growth.

In addition to promoting cell division and differentiation, Bp also regulates lignin biosynthesis in the stem, and represses several genes involved in lignin biosynthesis to prevent irreversible differentiation of cells in the stem (Douglas et al. 2002; Mele et al. 2003). Consistent with this, the short internodes of bp mutants are more lignified than wild type. The basal solid internodes in tin are stiff and in severely stunted plants they are curved and twisted or deformed and appear to be more lignified (Kebrom Personal observation). Duggan et al. (2002) suggested that high photothermal quotient (PQ) may be responsible for the induction of stunting by high irradiance or elevated CO2. In fact, availability of sucrose and elevated CO2 increased lignification in Arabidopsis seedlings and stem of hybrid poplar (Populus tremula × alba) respectively (Rogers et al. 2005; Richet et al. 2012). The developmental defects of internodes in stunted tin plants appear to be similar to those of bp, Rs1 and osh15 mutants. The regulation of BP by plant hormones provides additional evidence for the similarities between tin and bp mutants.

![Fig. 3. Internode elongation but not initiation is affected in stunted tin plants line 492. (a) Normal (left) and stunted (right) tin plants. (b) Internodes of the normal (left) and stunted (right) plants. Arrows indicate nodes. The number of internodes in stunted plants is equal to or slightly higher than normal plants. However, their length is much reduced and sometimes appears twisted, curved or deformed. In addition the development of the reproductive shoot apex of stunted plants is retarded. Not only is internode elongation inhibited, but sheath length might also be reduced in stunted plants, as shown by the absence of distance between ligules of successively formed leaves in the stunted plants.](image)
Hormone application studies indicated that stunting in tin is induced by ABA and ethylene and prevented by auxin (Inbal and Atsmon 1983). Further, the developmental defects of tin appear to be due to imbalance in hormone level and the level of ABA in line 492 is higher (Inbal and Atsmon 1983). The biosynthesis and localisation of hormones implicated in stunting such as auxin, ethylene and ABA are regulated by environmental factors. We note that in Arabidopsis, the BP gene is repressed by ABA and promoted by auxin (Soucek et al. 2007), both with opposite effect on stunting in tin. Stunting induced by ABA in tin is counteracted by AVG suggesting that ABA induces stunting through increased biosynthesis of ethylene (Inbal and Atsmon 1983). However, the relationship between ethylene and ABA is complex, depending on developmental stage and site of action (Beaudoin et al. 2000). Since ethylene has both growth promoting and inhibiting effect (Pierik et al. 2006) and both internode elongation under normal developmental condition and internode inhibition under stunting inducing condition are exhibited by tin, ethylene level could be at the centre stage of the tin phenotype and may involve changes in the expression of the KNOX and BELL-like transcription factors. In fact, ethylene regulates the expression of KNOX genes (Hamant et al. 2002; Osnato et al. 2010). Therefore, stunting may involve regulation by more than one distinct mechanisms acting together or independently. Further systematic studies of the role of plant hormones as well as genes involved in internode elongation such as the KNOX and BELL may reveal the physiological basis of stunting in tin.

Future research directions in tin

The wheat tin mutant provides an unprecedented opportunity to study several aspects of plant/crop physiology and cereal development including tillering, stunting, internode initiation and elongation, and their regulation by genetic, environmental and hormonal factors. It also provides an opportunity to study the effects of photoassimilate partitioning during early stages of plant growth and the effects of these on reproductive growth and yield. Tin is also being used in breeding because of advantages it may offer to improve grain yield and adaptation to specific environments but there remain uncertainties of effects of genetic background, expression of some desirable features in crop canopies and whether genotype specific management practices may need to be developed to ensure the changed architecture associated with tin results in high yields. More information is also required on the environmental conditions and agronomic practices where lines with tin are likely to be advantageous. As tin is being used in breeding it is important to understand the expected probability with which stunting will occur in field environments and the environmental and genetic factors that lead to stunting. Uncoupling stunting from reduced tillering is clearly going to be the optimal solution. The onset of both tiller inhibition and stunting in tin is associated with transition of the shoot apex from vegetative to flowering phase, which is promoted by long days. Once transition to flowering is induced, internodes start to develop in tin, marking the onset of inhibition of tillering. The initiation of internode elongation appears to be under the regulation of the KNOX/BELL-GA2ox1 pathway. Once internodes are initiated in tin, their normal development leads to a normal plant with ‘gigas’ leaves and spike and reduced tillering. However, stunting may occur under low night temperatures and long days, in which case, the internodes may fail to elongate properly. In Arabidopsis, maize and rice internode elongation is mainly regulated by genes belonging to the KNOX and BELL family of transcription factors. The defects in internode development in bp, Rs1 and osh15 mutants of Arabidopsis, maize and rice show many similarities to the developmental defects of internode elongation in stunted plants in tin. Fortunately, the wheat orthologs of many of the KNOX and BELL transcription factors mentioned in this paper are cloned (Takumi et al. 2000; Morimoto et al. 2009; Mizumoto et al. 2011). Since both precocious internode initiation and subsequent elongation appears to be the developmental defects of tin, the information on the regulation of internode initiation and growth identified in other species and reviewed in this paper provide an excellent starting point for studying the physiological, molecular and genetic basis of stunting in tin and will facilitate the cloning of the Tin and stunting genes.

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