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The fission yeast mitotic activator cdc25 and sucrose induce early flowering synergistically in the day-neutral *Nicotiana tabacum* cv. Samsun

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Summary

• Here, the tobacco (*Nicotiana tabacum*) day-neutral (DN) cv. Samsun transformed with the *Schizosaccharomyces pombe* mitotic activator gene *Spcdc25* was used to study the onset of flowering.

• Wild type (WT) and cdc25 plants were grown from seeds *in vitro* until they were 20 cm high. Apical and basal nodes were then subcultured repeatedly and the regenerated plants were used to document time to flowering and the number of leaves formed before flowering. Three sucrose treatments (3, 5 or 7% (weight/volume)) were used and measurements of leaf endogenous soluble carbohydrates were performed.

• In the 3% treatment, cdc25 plants flowered but WT plants did not. The higher sucrose treatments enabled WT flowering; two-thirds of the plants flowered at 5%, while all plants flowered at 7% sucrose. However, in all treatments, cdc25 plants exhibited significantly earlier flowering and fewer leaves compared with wild type. Remarkably, a typical acropetal flowering gradient in WT plants did not occur in cdc25 plants. In cdc25 leaves, there were significantly higher amounts of endogenous sugars with a higher proportion of sucrose compared with WT.

• Our data demonstrate that *Spcdc25* expression and sucrose act synergistically to induce precocious flowering.

Key words: *cdc25* gene, cell cycle regulation, day neutral tobacco, flower induction, *Nicotiana tabacum*, *Schizosaccharomyces pombe*, sucrose.

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Introduction

Many studies on flower initiation have made use of obligate photoperiodically sensitive plants, enabling floral mechanisms to be studied using treatments that result in either 100% or zero flowering (Bernier, 1988; Macháčková *et al.*, 1993; Lejeune *et al.*, 1994; Corbesier *et al.*, 2004). However, recently even more effort has been devoted to the facultative long-day plant *Arabidopsis thaliana*. Here, the disadvantage of a quantitative floral response is far outweighed because genetic approaches to the flowering problem are possible using Arabidopsis as a model. Thus, much is now known about the genes that regulate floral induction and about floral genes expressed in the shoot apical meristem (Boss *et al.*, 2004; Blazquez *et al.*, 2006). Meristem identity genes respond to the floral stimulus by switching the fate of the shoot apical meristem (SAM) from vegetative to floral. Among them, *LEAFY* (*LFY*) expression is necessary to specify a meristem as floral (Jack, 2004). The 'unseen' changes that commit a SAM to become floral have been termed 'floral evocation' (Evans,

1971). This is followed by the expression of organ identity genes downstream that control the fate of the floral whorls, the appearance of which marks the beginning of floral realization.

Much less is known about the regulation of flowering in true day-neutral (DN) plants which, by definition, flower equally well under short-day and long-day conditions and in which *LFY* is expressed at similar levels in vegetative and floral meristems (Kelly *et al.*, 1995). Autonomous developmental events regulated by endogenous signals must prevail in DN plants (Bernier *et al.*, 1981, 1993; McDaniel, 1992). Pioneer work on DN plants established that flowering depended on the SAM acquiring floral competence as it passed from the juvenile to the adult state. For example, *Impatiens parviflora* flowered only when the eighth leaf pair became vascularized (Hughes, 1965). Also, when juvenile scions of *Larix leptolepis* (Japanese larch) or *Larix decidua* (European larch) were grafted onto mature plants, and examined 1 yr later, only one out of 56 surviving scions flowered in Japanese larch but none of the European larch scions produced cones (Robinson & Wareing, 1969). Conversely, Lang (1965) argued that differences between juvenile and mature plants were a result of the ability of a plant to attain the induced state and produce florigen. More detailed work established that DN tobacco (*Nicotiana tabacum*) flowered after initiating a specific number of leaves. If a plant that had already produced 20 leaves was cut at its stem base and replanted, it made another 20 leaves before flowering, exactly the same number of leaves present in plants that were left undisturbed (Dennin & McDaniel, 1985). Hence, even if leaves of DN plants can make the floral stimulus regardless of day length, the SAM must become temporally competent to flower.

In DN plants, Gebhardt & McDaniel (1991) found that more apical leaves were more effective in inducing flowering than more basal ones nearer to the root system. They hypothesized that a floral inhibitor produced by the root system was sufficient to prevent the SAM from making an inflorescence until further leaves were formed, thereby displacing the SAM from the field of root inhibition (Gebhardt & McDaniel, 1991). The general picture is therefore of a floral stimulus that is able to offset inhibitors produced by the roots, by inducing flowering in developmentally competent SAMs.

Extensive grafting experiments in tobacco established that the floral stimulus is graft-transmissible (Zeevaart, 1962; Chailakhyan & Khazakhyan, 1974a,b; Lang, 1989). However, repeated failures to identify florigen led to the hypothesis that the floral stimulus is multicomponent in nature and that sugars are important subcomponents of it (Bernier, 1988). In inducible plants, an inductive photoperiod can cause a rapid increase in sucrose concentrations in leaf exudates (Houssa *et al.*, 1991; Lejeune *et al.*, 1991) and a rapid and, in some cases, transient accumulation of sucrose in the SAM (Bodson & Outlaw, 1985; Bernier *et al.*, 1993; Corbesier *et al.*, 1998). Interestingly, the addition of sucrose can rescue the late-flowering phenotype of several Arabidopsis mutants (Roldan *et al.*, 1999). More recently, Huang *et al.* (2005) discovered *FLOWERING LOCUS T* (*FT*), which encodes an mRNA that is transported from the leaf to the apex. The consensus view is that FT is a component of florigen and that it regulates the meristem identity gene *APETALA1* (*AP1*) which, together with *CAULIFLOWER* (*CAL*) and *LEAFY* (*LFY*), commits Arabidopsis SAMs to become floral (Aksenova *et al.*, 2006).

To accommodate all organs of each floral whorl, SAMs become larger than vegetative SAMs (Nougarède *et al.*, 1987, 1991). The increase in meristem size immediately before floral realization is the result of shorter cell cycles (e.g. Miller & Lyndon, 1976). Lang (1965) suggested that derepression of floral genes in the SAM might require not only the arrival of the floral stimulus but also stimulation of DNA replication in the SAM. One of the earliest detectable events of floral evocation was a mitotic burst observed in SAMs in the long-day plants *Sinapis alba* and *Silene coeli-rosa* (Bernier *et al.*, 1967). In the latter, the transient flurry of mitoses and an increase in the rate of DNA replication occurred 8 d before the start of floral realization (Francis & Lyndon, 1978; Ormrod & Francis, 1986). Moreover, in *Antirrhinum*, rapid bursts of cell division in the floral meristem were suppressed in nonflowering *floricaula* (*flo*) mutants (*FLO* is homologous to Arabidopsis *LFY*). It was suggested that developmental control genes regulate cell division in the prefloral SAM (Vincent *et al.*, 1995; Doonan, 1998; Bernier & Perilleux, 2005).

Previously, we showed that, in tobacco, flowering occurred earlier (by *c.* 30 d compared with wild type (WT)) in tobacco plants expressing the *Schizosaccharomyces pombe* mitotic inducer gene *Spcdc25* (Bell *et al.*, 1993). However, this was not followed by a detailed temporal study of time to flowering in T1 and their descendants. Our aim was to fill this gap by undertaking a thorough analysis of flowering time in plants that developed from cultured nodes of *Spcdc25*-expressing plants (referred to hereafter as 'cdc25 plants'). Also, given several reports suggesting sucrose as a likely component of the floral stimulus, we examined the effects of sucrose treatments on flowering and measured the concentrations of endogenous sucrose, glucose and fructose in the leaves of WT and cdc25 plants.

The data reported here show that, compared with WT, *Spcdc25* expression results in precocious flowering and a uniform floral response from both apical and basal node segments. Moreover, sucrose and *Spcdc25* expression synergistically shortened time to flowering.

Materials and Methods

Plant material

We used wild-type *Nicotiana tabacum* L., cv. Samsun, and two lines of this cultivar transformed with *cdc25* cDNA from *Schizosaccharomyces pombe* under the 35S CaMV promoter

and the nopaline synthase terminator (designated lines A and C) (Bell *et al.*, 1993).

Plant cultivation

Both WT and cdc25 seeds were surface-sterilized and germinated on solid hormone-free Murashige–Skoog (MS) medium (Murashige & Skoog, 1962) with 3% sucrose at 25°C, with a 16-h photoperiod and irradiance of 240 μmol m^{-2} s^{-1}. Three weeks after sowing *in vitro* on medium with 3% sucrose, the young seedlings were transferred into large Erlenmeyer vessels with 3, 5, 7 or 9% (weight/volume (w/v)) sucrose concentrations. These plants were allowed to grow to a height of 20 cm. Two positionally different node segments, hereafter referred to as (1) the basal segment (the second node without a leaf) and (2) the apical segment (an apex with leaves smaller than 2 cm), were then isolated and further cultivated on the MS medium with 3, 5, 7 or 9% sucrose. They were again allowed to develop to a height of 20 cm, and then the basal segments (excised from plants derived from basal segments) and apical segments (excised from apical segment-derived plants) were repeatedly subcultivated onto fresh medium. Time to flowering was recorded as the time from transfer of 3-wk-old seedlings into large Erlenmeyer vessels until flower formation.

Root system evaluation

At the time of segment excision, which was carried out when the plants had grown to an approximate height of 20 cm, the whole root system was removed and weighed.

Determination of the nonstructural saccharide (NSS) content

Samples of fully developed leaves from the 20-cm-high plants (60 mg fresh weight per sample) were freeze-dried and homogenized. They were then boiled with 80% methanol, the solvent was evaporated, and the residue was dissolved in Milli-Q ultra-pure water (Millipore, Bedford, MA, USA). The content of extracted soluble NSS was determined using high-performance liquid chromatography (HPLC) with refractometric detection; column IEX Pb form (Watrex, Prague, Czech Republic). For details, see Lipavská *et al.* (2000).

Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis

Total RNA was isolated from 1-month-old seedlings (WT and lines A and C) using the TRI-Reagent isolation protocol (Sigma Aldrich, St Louis, MO, USA). RNA integrity was checked using RNA denaturing agarose gel electrophoresis (Mašek *et al.*, 2005) and quantified at $\lambda = 260$ nm. Total RNA (15 μg) was treated with DNaseI using the DNAfree kit (Ambion, Austin, TX, USA) in order to eliminate genomic DNA contamination. Purified RNA (5 μg) was reverse-transcribed using SuperScriptII RNAseH$^-$ Reverse Transcriptase (Invitrogen, Paisley, UK) according to the manufacturer's instructions. An oligo dT primer was used for cDNA synthesis. A volume of 2 μl of the RT reaction mixture was subjected to PCR amplification of *Spcdc25* templates using gene-specific primers (forward primer: 5′-TTAGGTCCCCTTCTCCGATG-3′; reverse primer: 5′-TCAATGAGTCCTCCTTCACG-3′). PCR was carried out using the High Fidelity PCR Master system (Roche, West Sussex, UK) according to the manufacturer's instructions. After 40 cycles of amplification (initial template denaturation at 94°C for 2 min, denaturation at 94°C for 20 s, primer annealing at 53°C for 30 s, extension at 68°C for 1 min, and final extension at 72°C for 7 min), aliquots of the PCR samples were run on 2.5% Tris–acetate–EDTA (TAE) agarose gels and visualized by ethidium bromide staining. For comparison of cDNA synthesis efficiency, constitutively expressed transcripts of the tobacco actin gene *NtActI* (accession no. X63603) were detected in independent PCR reactions from RT samples using the same amplification conditions as for *Spcdc25* (forward primer: 5′-AAGCACCTCTTAACCCGAAGG-3′; reverse primer: 5′-CACCGATGGTAATCACTTGACC-3′). To exclude the possibility of DNA contamination, every experiment was supplemented by RT-PCR controls without the addition of reverse transcriptase. The identity of *Spcdc25* amplification products was checked by restriction fragment analysis.

Statistical analysis

A one-way ANOVA was used for statistical comparison of data (Tukey–Kramer and Kruskal–Wallis tests; $P < 0.05$). In Figs 2, 3, 5 and 6 (see below), bars indicate standard deviations and different letters over columns indicate statistically significant differences between means.

Results

Confirmation of *Spcdc25* expression

Of eight independent *Spcdc25*-transformed tobacco lines, four lines were selected for further studies (lines A, B, C and F). *In vivo*, each of the four lines flowered earlier than WT and the number of leaves formed before flowering ranged between 12 and 13, compared with 34–35 in WT. Then we selected two transformed lines of cdc25 plants, A and C (Suchomelová *et al.*, 2004a,b), and confirmed the expression of *Spcdc25* at the RNA level by RT-PCR. Line C exhibited a higher level of *Spcdc25* mRNA than line A (Fig. 1); this pattern of differential transcript levels was found in several replicate experiments. The results are consistent with more cytokinin-independent vegetative shoot formation *in vitro* in line C compared with line A (Suchomelová *et al.*, 2004b). Line C (hereafter designated 'cdc25 plants') was used in subsequent experiments.

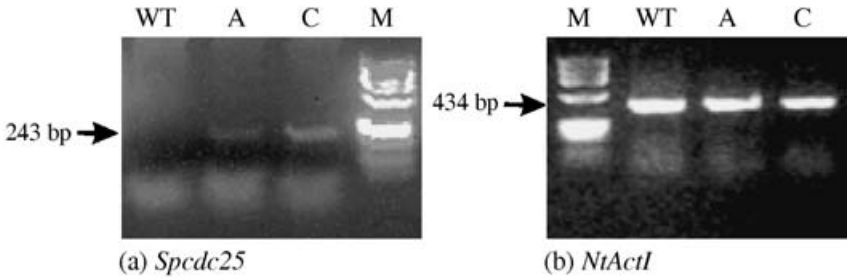

Fig. 1 Detection of *Schizosaccharomyces pombe Spcdc25* transcripts in transgenic tobacco (*Nicotiana tabacum*) plants. Semi-quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) analyses of (a) *Spcdc25* transcripts and (b) the constitutively expressed actin gene *NtActI* were performed. RNA was isolated from leaves of 1-month-old seedlings. WT, wild-type nontransformed plant material; A, *Spcdc25*-expressing plants, line A; C, *Spcdc25*-expressing plants, line C; M, DNA molecular weight marker IX (Roche).

cdc25 plants flower precociously

When plant material from cdc25 plants was regenerated on 3% sucrose under *in vitro* conditions using nodal segments, time to flowering was found to be progressively shorter with increasing number of subcultivations. This trait was also shown by line A and by two other independently transformed lines (B and F; see Materials and Methods). By contrast, flowering was not observed for WT on 3% sucrose.

A detailed investigation of this phenomenon was carried out using precisely defined plant segments. Both WT and cdc25 plants were subcultured repeatedly. In the 3% sucrose treatment, cdc25 plants regenerated from apical and basal segments had average times to flowering of 233 and 208 d, and initiated 65 and 53 leaves, respectively. However, WT did not flower on 3% sucrose even after 350 d, producing 233 and 155 leaves in apical and basal segment-derived plants, respectively (Figs 2, 3). After 350 d in culture, WT plants of height 20 cm were removed from the flasks and were further grown in soil. Remarkably, these WT plants formed the same number of leaves before flowering as did WT plants derived from conventional seed germination (Fig. 4b). For comparison, Fig. 4(a) shows a seed-derived cdc25 plant which, compared with WT, flowered earlier and formed fewer leaves.

Spcdc25 and sucrose act synergistically

In the 5% treatment, the plants derived from apical and basal segments of cdc25 plants flowered after 182 and 174 d, respectively (Fig. 2), while the mean leaf number was 33.1 and 26.1, respectively (Fig. 3). Two-thirds of WT plants flowered in the 5% treatment, with average times to flowering of 231 and 303 d, respectively, while the total numbers of leaves were 60.8 and 91.3, respectively (Figs 2, 3). These flowering times were much longer than those of cdc25 plants in the 3 and 5% treatments. Moreover, in cdc25 plants there were no significant differences in number of leaves formed and days to flowering between the basal and apical nodes (Figs 2, 3). Hence, the acropetal floral competence of WT plants was not found in the cdc25 plants.

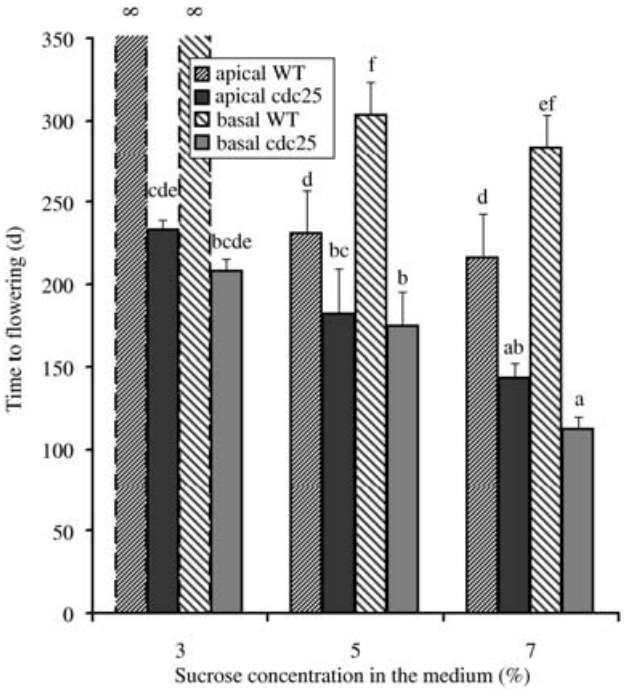

Fig. 2 Precocious flowering of cdc25 compared with wild-type (WT) tobacco (*Nicotiana tabacum*) as affected by sucrose supply. The mean time to flowering (days ± SE) in WT and cdc25 plants (line C) in culture medium supplemented with 3, 5 or 7% sucrose was determined. The plants were repeatedly cultivated either from apical or from basal segments (n = 3–12). '∞' indicates that WT plants did not flower on 3% sucrose even after 350 d in culture. Four of 12 WT plants had not flowered on 5% sucrose by the end of the experiment (330 d) (data not included in the figures). Statistically significant differences between variants are indicated by different letters above columns.

In the 7% sucrose treatment, time to flowering for cdc25 plants further decreased to 143 and 113 d for plants derived from apical and basal segments, respectively (Fig. 2), while leaf numbers were only 22 and 19.2 (Fig. 3). WT plants derived from apical and basal segments flowered after 216.1 and 283.5 d, respectively (Fig. 2), and leaf numbers were 53.4 and 81.5, respectively (Fig. 3). The rate of leaf initiation was

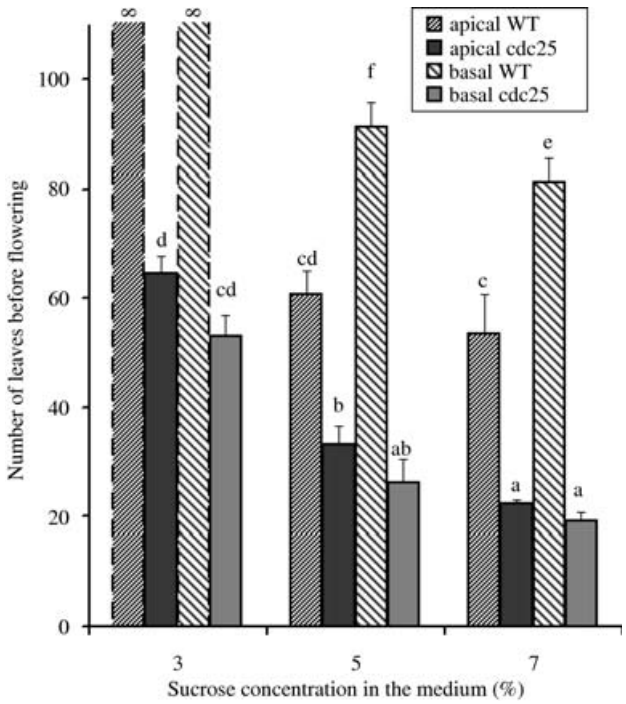

Fig. 3 cdc25 tobacco (*Nicotiana tabacum*) plants make fewer leaves before flowering than wild type (WT) in all sucrose treatments. The mean number of leaves (± SE) before flowering in WT and cdc25 plants (line C) in culture medium supplemented with 3, 5 or 7% sucrose was determined. The plants were repeatedly cultivated either from apical or basal segments (n = 3–12). '∞' indicates that WT plants did not flower on 3% sucrose even after 350 d in culture. Four of 12 WT plants had not flowered on 5% sucrose by the end of the experiment (330 d) (data not included in the figures). Statistically significant differences between variants are indicated by different letters above columns.

slower in cdc25 plants compared with WT (Table 1). Hence, time to flowering in cdc25 plants was shorter in the 7% sucrose treatment than at 3 or 5%, and was substantially shorter compared with that in WT plants in the corresponding sucrose treatments (Fig. 2). In WT plants, increasing the sucrose from 5 to 7% had little impact on time to flowering. Further, in WT plants, an acropetal flowering gradient was also evident under these conditions, while cdc25 plants derived from apical and basal segments flowered equally well (Fig. 3). Raising the sucrose concentration to 9% resulted in poor growth of plants, probably because of severe osmotic stress, which did not allow an evaluation of time to flowering.

Overall, the data are consistent with a synergistic effect of *Spcdc25* expression and increased sucrose on the switch to flowering.

Root dry weight accumulation was lower in cdc25 plants

In the 5 and 7% treatments, root dry weight was significantly lower in cdc25 plants compared with WT plants (Fig. 5). Repeated root removal, however, had no effect on flowering onset (M. Teichmanová, unpublished data). Hence, a correlation did not exist between inhibition of flowering and the presence of a root system.

Endogenous sugars were higher in cdc25 plants with higher sucrose:hexoses ratios

It was of further interest to assess the effect of increasing the concentration of exogenous sucrose on the endogenous concentrations of sucrose, glucose and fructose in the leaves (Fig. 6). The increase in exogenous sucrose from 3 to 5% did not result in any significant difference in the concentrations of

Fig. 4 cdc25 tobacco (*Nicotiana tabacum*) plants form fewer leaves at the time of flowering. (a) A cdc25 plant (line C); (b) a wild-type (WT).

Table 1 Rate of tobacco (*Nicotiana tabacum*) leaf initiation as affected by *Schizosaccharomyces pombe Spcdc25* expression

	cdc25						WT					
	No. of leaves at time of flowering		Time to flowering (d)		Rate of leaf initiation (d^{-1})		No. of leaves at time of flowering		Time to flowering (d)		Rate of leaf initiation (d^{-1})	
Sucrose (%)	Apex	Base	Apex	Base	Apex	Base	Apex	Base	Apex	Base	Apex	Base
3	65	53	233	208	0.27	0.25	223	154	350	350	0.66	0.44
5	33	26	182	175	0.18	0.15	60	90	231	303	0.26	0.3
7	22	20	143	112	0.15	0.17	53	81	216	283	0.24	0.29

The number of leaves, the time to flowering and the calculated rate of leaf initiation were determined in wild-type (WT) and cdc25 plants (line C) derived from apical (apex) and basal (base) node segments cultured on 3, 5 or 7% sucrose ($n = 3–12$).
Notably, control plants did not flower on the medium with 3% sucrose even when cultivation was prolonged for 350 d and only two-thirds of WT plants on 5% sucrose initiated flowers; the data in the table pertain to those plants.

water-soluble carbohydrates, sucrose, glucose and fructose either for WT or for cdc25 plants. However, in each treatment, the content of sugars was significantly higher in cdc25 plants, with glucose and fructose prevailing in the spectrum. Also, while sucrose was detected in cdc25 plants at all three exogenous concentrations, it was barely detectable in WT plants.

Discussion

Spcdc25 expression results in reduced time to flowering and a decreased rate of leaf initiation. Moreover, *Spcdc25* expression and increased exogenous sucrose had a synergistic effect in shortening the time to flowering as well as in reducing the number of leaves formed before flowering.

Spcdc25 phosphatase in fission yeast, and also when introduced to *Nicotiana plumbaginifolia*, dephosphorylates Cdc2 at G2/M, enabling cells to enter mitosis (Nurse, 1990; Zhang *et al.*, 2005). Expression of *Spcdc25* induces small cell size in fission yeast (Russell & Nurse, 1986), in root meristems of tobacco (McKibbin *et al.*, 1998), and in tobacco BY-2 cells (Orchard *et al.*, 2005). However, cdc25 expression does not induce a shorter cell cycle in fission yeast (Russell & Nurse, 1986), in tobacco lateral roots, or in BY-2 cell cultures; a shortening of G2 is compensated by a lengthening of G1 (Russell & Nurse, 1986; McKibbin *et al.*, 1998; Orchard *et al.*, 2005). Given these well-established effects, we conclude that, in the current work, *Spcdc25* is functioning in plants as a mitotic inducer. In previous work, we established that expression of *Spcdc25* in tobacco BY-2 cells led to precocious cyclin-dependent kinase (CDKB) activity, but CDKA activity appeared to be unaffected (Orchard *et al.*, 2005). In WT BY-2 cells, CDKB peaks in mid G2 phase while CDKA activity is relatively constant (Sorrell *et al.*, 2001). We linked the earliness of CDKB activity, which peaks in early S phase instead of mid G2, to early mitotic activation, a shortened G2 phase and a small cell size at mitosis (Orchard *et al.*, 2005). We have also observed a shortened cell length in epidermal cells of root apical meristems of Arabidopis (D. Francis *et al.*, unpublished data). In the current work, *Spcdc25* may have the effect of establishing a premature cluster of mitotic divisions in the cultured node which develop competence to respond to the floral stimulus. However, this is not to deny that the same type of cell cycle activity occurs at the time of flowering in nodes of WT plants cultured on 5 or 7% sucrose. We wish to emphasize that, if our model is correct, on 5% sucrose it is the timing of mitotic activity that is earlier in *Spcdc25* plants compared with WT, as opposed to the induction of a cell cycle response *per se*.

A long-standing hypothesis is that the floral stimulus is propagated in the SAM via cell division (Zeevaart, 1962; Lang, 1965; Ormrod & Francis, 1986). Also, one of the earliest reported events of floral evocation is more cells in mitosis, which precedes by many hours the earliest growth changes affecting the initiation of the first reproductive structures (Bernier, 1988; Jacqmard *et al.*, 2003). Preliminary work using 3% sucrose failed to detect any increase in the mitotic index in vegetative buds in cdc25 plants compared with WT (P. Mašková *et al.*, unpublished). On this basis, we suggest that *Spcdc25* induces premature entry of cells into mitosis rather than a shortening of the cell cycle.

Cytokinins are important regulators of floral competence in the SAM (e.g. Bernier & Perilleux, 2005). Notably, *Spcdc25* expression can replace cytokinins to induce vegetative shoot formation in *de novo* organogenesis (Suchomelová *et al.*, 2004b), to dephosphorylate plant CDK (Zhang *et al.*, 2005) and to drive cells into mitosis prematurely (Orchard *et al.*, 2005). Collectively, these observations indicate a critical interface between cytokinins required for SAM development and cytokinins required for activating more cells into mitosis. Our data are consistent with *Spcdc25* expression at least partly replacing a critical cytokinin requirement to establish florally competent SAMs. Moreover, cdc25 plants exhibited a lower cytokinin content compared to WT (our unpublished data).

In DN plants, time to flowering is affected by a balance between floral stimulus from leaves and possible inhibitors from the root and the time taken for SAMs to become florally

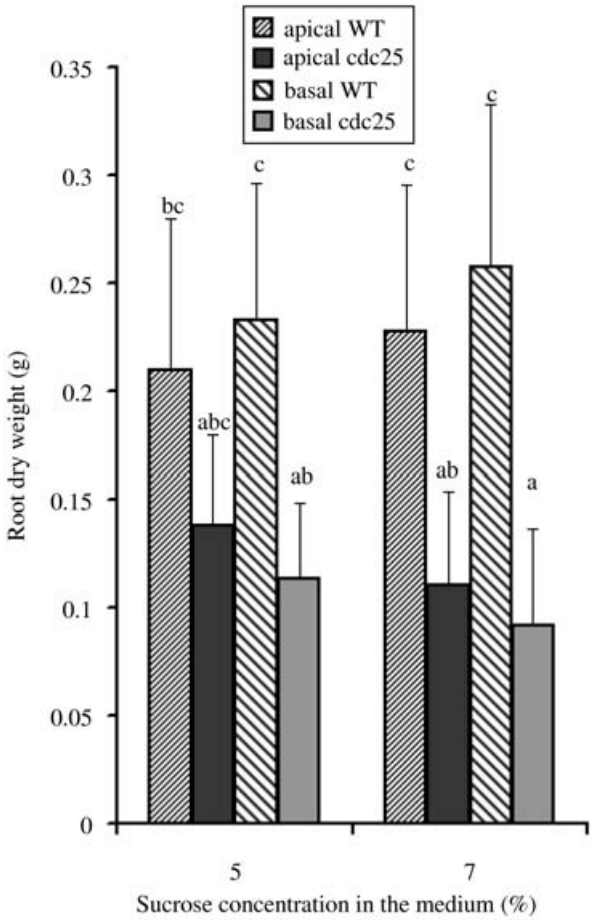

Fig. 5 cdc25 tobacco (*Nicotiana tabacum*) plants exhibit a less developed root system compared with wild type (WT). Dry weights of the root system of WT and cdc25 plants (line C) in culture media supplemented with 5 and 7% sucrose were determined. The root system was sampled at the time of segment subculture (mean values ± SE; $n = 5–11$). Statistically significant differences between variants are indicated by different letters above columns.

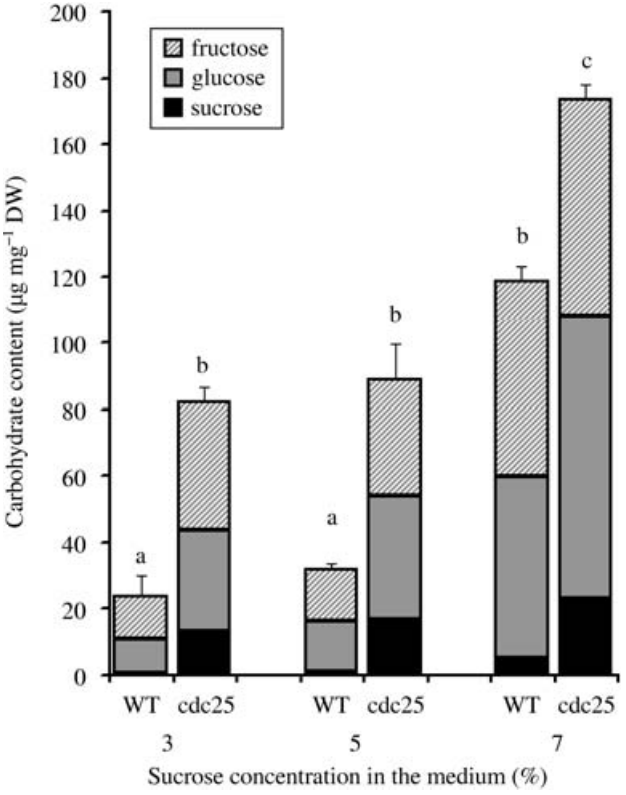

Fig. 6 cdc25 tobacco (*Nicotiana tabacum*) plants exhibit higher carbohydrate contents with a larger proportion of sucrose compared with WT. Contents of endogenous nonstructural carbohydrates (hatched bars, fructose; grey bars, glucose; black bars, sucrose) in the leaves of WT and cdc25 plants (line C) after 77 d of cultivation are shown (mean values ± SE; $n = 3–5$). Statistically significant differences between variants are indicated by different letters above columns. DW, dry weight.

competent (Tran Thanh Van, 1973; Tran Thanh Van *et al.*, 1974; McDaniel *et al.*, 1996). Thus, premature flowering in cdc25 plants could be because of a strengthened floral stimulus or earlier floral competence, or both. We reject the idea of root inhibition of flowering because cdc25 plants flowered equally well with or without a root system.

If the floral stimulus is strong enough and transmitted regardless of developmental time, then flowering soon after germination might be predicted providing that the SAM was florally competent. With some exceptions, for example *Pharbitis nil* and *Chenopodium amaranthe* (Macháčková *et al.*, 1993), most plants must go through a juvenile-to-mature phase before flowering can occur. Here, cdc25 plants were found to have a shortened time to flowering, which must mean that the SAM acquires floral competence more rapidly than in the WT.

In photoperiod-sensitive plants, carbohydrates have a critical function in the floral transition (Corbesier *et al.*, 1998; Roldan *et al.*, 1999). However, reports concerning the influence of carbohydrates on flowering in DN plants, for example in tobacco (Konstantinova *et al.*, 1972, 1976) and in tomato (Dielen *et al.*, 2004), are rather scarce. Tran Thanh Van (1973), in a study of tobacco, showed that epidermal thin layers (ETLs) from floral plants could make floral buds in culture but only vegetative buds would form on vegetative ETLs. Our results are distinctly different, because in cdc25 plants nodal segments regenerated plants that formed flowers regardless of their developmental state. Again, this suggests that cdc25 plants develop early floral competence.

The determination of endogenous carbohydrate concentrations in leaves revealed that cdc25 plants have higher concentrations of glucose and fructose compared with WT, while sucrose was detected in cdc25 plants but was barely detectable in WT. Therefore, endogenous sugar concentrations become a factor in the flowering of cdc25 plants. Several authors have reported dramatic increases in the amount of sucrose reaching the apex after induction in photoperiodic plants. As this change long preceded any morphological events, they suggested a message-like role for sucrose in flowering (Lejeune *et al.*, 1993; Corbesier *et al.*, 1998; Dielen *et al.*,

2001). Essentially this was also a conclusion drawn from flowering responses of *P. nil* in culture (Durdan *et al.*, 2000; Parfitt *et al.*, 2004). Whether the role of sugars in DN plants is a morphogenetic signal or just a nutritive one is the subject of an on-going debate (see Francis & Halford, 2006).

Roldan *et al.* (1999) proposed that sucrose could promote flowering by regulating the expression of the flowering repressor *FLOWERING LOCUS C* (*FLC*), a key regulator of the autonomous flowering regulatory pathway (Putterill *et al.*, 2004), and probably also of day-length controlled pathways (Noh *et al.*, 2004). Note that *Spcdc25* expression and exogenous sucrose had a synergistic effect in shortening the time to flowering. Elucidation of the extent to which this mitotic activator affects sucrose transport to SAMs would be very worthwhile.

cdc25 plants were found to have less developed root systems when evaluated on a dry weight basis, which would reduce levels of putative floral inhibitors from the root (Fig. 5). This might be why the acropetal flowering response present in WT was abolished in cdc25 plants. However, as already discussed, cdc25 plants flowered equally well with or without a root system. In addition, the rate of leaf initiation was slower in cdc25 plants compared with WT under our given experimental conditions (Table 1). Thus, earlier flowering in cdc25 plants cannot be ascribed to faster growth rates of above-ground regions.

Surprisingly, the acropetal floral potential (McDaniel & Hartnett, 1993) observed when younger as opposed to older nodes were cultured was not evident in cdc25 plants (Figs 2, 3). The consensus view is that the floral gradient results from integrated signals of numerous chemical components. Using a late-flowering mutant, *uniflora*, Dielen *et al.* (2001) identified sucrose, cytokinins and nitrogenous nutrients that promoted the floral transition in day-neutral tomato. Corbesier *et al.* (2002) reported that the carbon:nitrogen (C:N) ratio in phloem sap increased during inductive treatments and the inequality in C:N supply may be important at floral transition in *S. alba* and Arabidopsis. Much more sucrose was detected in the leaves of cdc25 plants but hexoses were the most abundant water-soluble carbohydrates.

We report for the first time that *Spcdc25* expression and sucrose act synergistically to cause early flowering. We suggest that expression of *Spcdc25* in the SAM renders it cytokinin independent, as it does in tobacco cells in culture (Orchard *et al.*, 2005) as well as in *de novo* shoot formation (Suchomelová *et al.*, 2004b), and that sucrose interacts with a florally competent SAM. *In planta*, we hypothesize that in DN plants activation of more cells to enter mitosis in the SAM is an important mechanism for acquisition of floral competence.

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