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Relationship between sugar signalling, strigolactones and branching in *Arabidopsis thaliana*

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List of abbreviations

ABA	abscisic acid
AKIN10	*Arabidopsis* protein kinase KIN10
AM	arbuscular mycorrhizal
AtD27	Arabidopsis ortholog of rice DWARF27
AUX	auxin
BRC	BRANCHED protein
BR	brassinosteroids
CCD	carotenoid cleavage dioxygenase
CK	cytokinin
Col-0	Columbia type 0
Cyc	cyclin
D14	*DWARF14*
DAS	days after sowing
ddH_2O	double distilled water
DELLA	repressors of GA-dependent processes
DL	day length
DMSO	dimethyl sulfoxide
DR5	auxin-responsive promoter
DSLR	digital single lens reflex
DW	dry weight
ext.	extended
FSPM	functional-structural plant modelling
fw	forward
G1	gap phase 1 (cell cycle)
G2	gap phase 2 (cell cycle)
G6P	glucose-6-phosphate
GA	gibberellic acid
Glc	glucose
glm	generalized linear model
GR7	synthetic strigolactone analog 7
GR24	synthetic strigolactone analog 24
Hex	hexose
HXK1	hexokinase 1
IAA	indole-3-acetic acid
KIN10	SNF1-related protein kinase catalytic subunit alpha KIN10
LD	long day
L*er*	Landsberg *erecta*
Luc	luciferase
M	mitosis phase (cell cycle)
Mal	maltose
MAX	more axillary growth
ms	main stem
MS	Murashige and Skoog medium
MQ	Milli-Q water
N	nitrogen
NaClO	sodium hypochlorite
P_i	inorganic phosphate
P	*P*-value
P450	*Arabidopsis* cytochrome 450
PAT	polar auxin transport
PCD	programmed cell death
PHYB	phytochrome B
PIF	phytochrome-interacting factor
PIN1	peptidyl-prolyl cis-trans isomerase
NIMA-	interacting 1
PL	parental line
PM	plasma membrane
R:FR	red:far-red (ratio)
rev	reverse
RGR	relative growth rate
RH	relative humidity
RT	room temperature
rubisco	Ribulose-1,5-bisphosphate carboxylase/oxygenase
S	synthesis phase (cell cycle)
SAM	shoot apical meristem
SAS	shade avoidance syndrome
SD	short day
SE	standard error
seq	sequence
SL	strigolactone
SLA	specific leaf area
Sor	sorbitol
SNF1	sucrose non-fermenting related kinase 1
SnRK1	SNF1-related protein kinase 1
STP1	sugar transport protein 1
Suc	sucrose
T6P	trehalose-6-phosphate
Taq Pol.	heat stable polymerase protein
TE buffer	buffer containing Tris and EDTA
TOR	target of rapamycin
TPP	T6P-phosphatase
TPS	T6P synthase
Tre	trehalose
TreH	trehalase
TreP	trehalose phosphorylase
TreT	trehalose glycosyltransferring synthase
TreY	maltooligosyl-trehalose Synthase
TreZ	maltooligosyl-trehalose trehalohydrolase
TS	trehalose Synthase
UDP-glc	uridine diphosphate glucose
wt	wild type
x_0	inflection point

Chapter 0 – Abstract

The presence and perception of strigolactones (SLs) as well as sugar signalling play a crucial role in shoot architecture. Both factors are noted for a significant impact on axillary bud break but little is known about the subsequent step, their influence on branch outgrowth. While a mutation in SL signalling is given in *max2*, synthesis of this plant hormone is said to be missing in *max1*, *max3* and *max4* and assumed to finally inhibit bud break through MAX2 signalling in the wt. A phenotypical analysis of the wild type (wt) Col-0, *max2-1* and *max3-9* should reveal the impact on shoot architecture with focus on branching patterns in terms of quantity in numbers and length under altered sugar metabolism. For this purpose intermittent extended nights inducing sugar starvation and trehalose (Tre) spraying, which simulates a sugar surplus through elevated trehalose-6-phosphate (T6P) concentrations was applied during vegetative stages. These results also revealed the importance of distinguishing bud break from branch growth and determining a transition threshold of these developmental stages. A higher total branch number in both mutants yet less total branch length in *max2-1* was observed, which also possessed a significantly different shoot biomass allocation. Surprisingly, both sugar signalling treatments tended to lower branch growth in a non-corresponding, insignificant way. Observations show an impact of mutations on carbon allocation within the shoot towards the main source (rosette) and indicate a SL leakage in *max3-9*. Furthermore, MAX2 signalling seems to buffer against changes in shoot development induced by extended night and additional pleiotropic effects of max mutations were found.

Chapter 1 - Introduction

Most plants like *Arabidopsis* possess a high plasticity to cope with variable environmental conditions, from which they cannot escape being sessile organisms in regulating meristem activity (Cheng *et al.* 2013), including branching among other developmental mechanisms (Brewer *et al.* 2013, Leduc *et al.* 2014). This comprises quality and quantity of root and shoot growth, in which auxin (AUX) is most widely involved (Shinohara *et al.* 2013). Every leaf bears an initially inactive pluripotent meristem in form of a bud in its axil, which has the potential to break and develop into a branch, depending on internal and external conditions (Booker *et al.* 2004, Bennett *et al.* 2006, Dun *et al.* 2012). These factors include several sugar and hormonal signalling molecules in interaction (Ferguson and Beveridge 2009, Eveland and Jackson 2011, Wang and Ruan 2013). Moreover, a suppression of bud break can depend on its position in the plant architecture due to apical dominance and correlative inhibition (Ferguson and Beveridge 2009). In *Arabidopsis* the functionality of *MORE AXILLARY GROWTH* (*MAX)* genes is presumed to inhibit this lateral shoot growth through MAX2 signalling (Bennett *et al.* 2006, Koltai 2011) and is targeted by SLs.

Meristems are the origin of plant organs after germination (Skylar *et al.* 2011), facilitating growth through steadily proliferating stem cells (Beveridge *et al.* 2003, Wahl *et al.* 2010, Eveland and Jackson 2011). Besides hormonal activity, light capture (Rameau *et al.* 2015), mitotic activity and cell division plant development also depends on the nutrient supply, such as carbohydrate levels and can be maintained by application of sugars (Skylar *et al.* 2011). The latter also participate in the adjustment of cyclin D (CYCD) gene expression (Eveland and Jackson 2011), whereby a loss of CYCD3, requiring both Cytokinins (CKs) and sugars, causes a decrease in branching (Leduc *et al.* 2014). Sugars not only act as carbon source and energy provider but also as signallers for gene expression (Lee *et al.* 2004, Mirnezhad 2011, Wang and Ruan 2013, Cordoba *et al.* 2014, Van den Ende 2014). While photosynthetically produced during light periods they are partially used up during night when respiration takes place (Bläsing *et al.* 2005, Wiese *et al.* 2007, Graf *et al.* 2010, Arias *et al.* 2014). Generally, levels of soluble sugar are kept stable, however stress factors such as shade or heat can cause a decrease (Lee *et al.* 2004). The first metabolised energy supply during starvation is starch before protein and fat consumption sets in (Lee *et al.* 2004). Besides this, autophagy as a second pathway can be initiated to provide energy at a sufficient level (Izumi *et al.* 2013).

A large part of Sucrose (Suc) from photosynthesis is exported from the mature leaves (source) to the sinks to avoid a feedback inhibition (Ruan 2012) enabling high nutrient mobilization (Wang and Ruan 2013), while starch concentrations locally increase (Martins *et al.* 2013) after Suc levels become oversaturated (Martins *et al.* 2013). Sugars and other osmotic substances can decrease the cell's osmotic potential at high concentrations in the apoplast and subsequently lower the turgor, which is essential for the process of cell growth (Wang and Ruan 2013). Nutrients and hormones, especially AUX (Wang and Ruan 2013) are also incorporated in the signalling network such as stress feedback (Eveland and Jackson 2011, Cordoba *et al.* 2014). Trehalose (Tre, α-D-glucopyranosyl-[1–1]-α-D-glucopyranoside) is a disaccharide composed of two linked Glc molecules (Reignault *et al.* 2001, Schluepmann and Paul 2009), similar to the chemical constitution of maltose (Mal, α-D-glucopyranosyl-[1–4]-α-D-glucopyranoside). Several synthesis pathways of Tre have been discovered: TS, TreY/TreZ, TreP, TreT, whereas the best known mechanism in plants sequentially takes place as follows: UDP-Glc + G6P → (by TPS) → T6P → (by TPP) → Tre + P_i (Schluepmann and Paul 2009, Martins *et al.* 2013, Wang and Ruan 2013, Matsoukas 2014). The intermediate product T6P is an essential controller for several sugar-depending processes, including growth and plastid metabolism (Arias *et*

al. 2014). Hence, Tre indirectly operates as an effective signaller through T6P, for which only low quantities are required (Stoller *et al.* 2013), which explains the low endogenous concentrations of both Tre and its precursor (Schluepmann *et al.* 2004, Schluepmann and Paul 2009) and indicates its signalling role operating on gene expression instead of directly acting as a protector against biotic and abiotic stress or as a provider of carbon or energy (Schluepmann *et al.* 2004, Stoller *et al.* 2013). Growth enhancement and support can be attributed to its essential signalling function on carbohydrate presence as well as inhibition of SNF1-related protein kinase 1 (SnRK1), which can diminish the growth process by signalling starvation status (Schluepmann and Paul 2009, Stoller *et al.* 2013, Van den Ende 2014). In commercial applications Tre is utilised to boost yield increase or to prevent yields loss in crops. Early spraying is claimed to improve vitality of the plant, while Tre usage in later stages promotes sugar dislocation to the seeds or fruits (Stoller *et al.* 2013).

SLs are terpenoid lactones deriving from the carotenoid pathway (Leyser 2008, Hayward *et al.* 2009). Up until now, more than 15 derivates have been discovered, usually consisting of four rings (A–D) (Xie *et al.* 2010), including the SL synthetic analogues GR7 and GR24 (Akiyama and Hayashi 2006). *MAX3*, *MAX4* and *MAX1* operate in series (Bennett *et al.* 2006) to generate the SL signalling molecule MAX2, which is needed to suppress the axillary bud outgrowth (Hayward *et al.* 2009). This scheme can be explained as follows in *Arabidopsis*: carotenoid molecule→(*AtD27)* β-carotene isomerase→(*MAX3)* CCD7→(*MAX4)* CCD8→carlactone molecule→(*MAX1*) P450→SL molecule→(*AtD14*) α/β-hydrolase + (*MAX2*) F-box protein→ response, thus inhibition of branching (Yamada and Umehara 2015) . Hence, a mutation in the max pathway results in a phenotype with excessive branching (Koltai 2012). It was shown that MAX2 is mainly present in the nucleus (Shen *et al.* 2007, Stirnberg *et al.* 2007), operates locally and is expressed in the entire plant (Stirnberg *et al.* 2007). It is essential for inhibiting bud break in every node, enhancing BRC1 synthesis (Leduc *et al.* 2014). In max mutants AUX levels are elevated based on higher *DR5* expression and SLs are therefore involved in AUX signalling (Hayward *et al.* 2009). Corresponding to higher AUX content, levels of PIN1 proteins are raised, therefore elevated auxin transport occurs in the main stem (ms) of max mutants (Bennett *et al.* 2006) and due to the metamer architecture of *Arabidopsis* (Coste *et al.* 2011) levels ought to be increased in branches as well. It is also assumed that SLs operate downstream of AUX (Agusti *et al.* 2011, Cheng *et al.* 2013) and concentrations of both hormones interact in a linked feedback regulation compensating for deviating syntheses of each other (Hayward *et al.* 2009). As a result, an accumulation of AUX in max mutants induces SL synthesis (Ferguson and Beveridge 2009, Hayward *et al.* 2009, Xie *et al.* 2010, Koltai 2011, Brewer *et al.* 2013). However, concentration of latter is lower in the xylem of these mutants (Brewer *et al.* 2013).

Hormones have a mutual effect on synthesis, sensitivity and transport (Cheng *et al.* 2013). CKs are known to have a major diminishing impact on *BRC1* expression (Dun *et al.* 2012, Brewer *et al.* 2013), enhance bud outgrowth (Hayward *et al.* 2009, Leduc *et al.* 2014) and contribute to interfascicular cambium increase (Brewer *et al.* 2013). Their concentration in the shoot of *Arabidopsis* max mutants is the same in comparison to the wt, though SL mutants react stronger to direct applications of cytokinin (CK) on buds (Dun *et al.* 2012). Light quality and quantity are also essential factors determining the branching pattern and therefore plant architecture in dependence on shade, day length, adjacent plants and other light capturing objects (González-Grandío *et al.* 2013, Leduc *et al.*, 2014, Rameau *et al.* 2015). In temperate LD plants like *Arabidopsis* the photoperiod also affects the vegetative shoot growth pattern (Beveridge *et al.* 2003).

There are two well-known models, which illustrate the key roles of AUX and SL in the bud break process to date (Bennett *et al.* 2006, Agusti *et al.* 2011, Dun *et al.* 2012, Brewer *et al.* 2013, Cheng *et al.* 2013, Shinohara *et al.* 2013, Leduc *et al.* 2014, Rameau *et al.* 2015), while the roles of sugars as primary energy, biomass provider and signaller in this mechanism have to be considered as well (Mason *et al.* 2014, Rameau *et al.* 2015). According to the second messenger theory SLs repress and CKs support bud break in an antagonistic manner and their balance is adjusted by AUX (Dun *et al.* 2012, Brewer *et al.* 2013, Rameau *et al.* 2015), enhancing SL synthesis and CK degradation via indole-3-acetic acid (IAA) (Ferguson and Beveridge 2009). On the other hand, a decrease in Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (PIN1) proteins implies a repression of auxin export from the buds (Bennett *et al.* 2006), which maintain dormancy (Dun *et al.* 2012, Mason *et al.* 2014). Besides multiple hormones, AUX and SLs affect the polar auxin transport (PAT) in the ms (Cheng *et al.* 2013, Leduc *et al.* 2014, Rameau *et al.* 2015). PIN1 proteins, actively transporting auxin, are prevented from degradation through IAA and are removed from the plasma membrane (PM) by SLs (Shinohara *et al.*, 2013), while gibberellic acid (GA) sustains their position (Rameau *et al.* 2015). However, the shoot apical meristem (SAM), growing leaves (Francis and Halford 2006) and buds not only serve as auxin sources (Hayward *et al.* 2009), but also act as competing sinks, that require sugar for energy and growth supply.

Since branching comprises the combination of bud break and elongation both stages have to be considered for the overall outcome. Besides the bud break releasing effect of low MAX levels or missing feedback response and enhanced AUX transport in mutants the second branching step could be boosted as well. The same outcome might apply to sugar

signalling alteration by Tre application. Effects of both factors individually and in interaction with emphasis on branch outgrowth were observed. By taking advantage of Tre signalling effects on growth with the objective of promoting bud break and therefore presumably a higher outgrowth count, levels of Tre in *Arabidopsis* were exogenously increased by frequent spraying in our study. On the assumption that occasional sugar starvations induce a reduction of axillary bud activity and consequently lower branching activity, we also applied intermittent extended nights on further plants during these conducted experiments supposing the opposite effect.

A difference between SL synthesis and SL signalling mutants in branching performance could not be ruled out. Due to the balancing effect of AUX on reduced levels of MAX3, if not absent, this deficiency might be compensated. Other pathways may enable bypassing directly to *MAX4* expression or further downstream. On the other hand, the omitted feedback response in max2 mutants creates higher SL levels. A MAX2 independent action of SLs was claimed, which prompted us to analyse how SLs contribute to an elongation responses. Seedling experiments supported the assertion of a MAX2 independent response to SL.

In this study the effect of a mutation in max2 and max3 on axillary bud break based on branch count and branch outgrowth by means of branch lengths were observed. This should also have indicated if additional altered sugar- and SL metabolism had additive, synergistic, or antagonistic effects in this respect. Furthermore, findings of this research should contribute to the calibration of the functional-structural plant modelling (FSPM) project, which computes and predicts plant growth based on seed characteristics and environmental input, by taking these results into account under given conditions. These phenotypical observations were grounded on visible, developmental stages in accordance with length thresholds, based on photo analysis.

Chapter 2 - Materials and methods

Plant Material: *Arabidopsis thaliana* (Col-0) wt (PL 15001), *max2-1* (PL 13011), *max3-9* (PL 13013) and starvation Luc reporter (PL 13026) in Col-0 background were used. The max3-9 mutation originated from Landsberg *erecta* (L*er*) plants, which were backcrossed over 8 reiterations with Col-0. Seeds were transferred to water agar (Duchefa Biochemie), stratified for 2 d at 4 °C, transferred for 1 d to 24 °C, sown individually in 5.9x5.9 cm pots on soil and arranged in rows (Σ 20-25 plants per 41x60 cm tray) to facilitate adequate, equal spacing and to avoid interactions within the rhizosphere. Branch analysis was done 76 DAS at an advanced branching stage and plant material was subsequently dried at 105 °C over 20 h for weight measurements.

Growth conditions: To provide a preferably controlled, consistent and favourable light- and temperature environment a climate room (Unifarm, Wageningen) with 12/12 h light cycle at 22/17 °C, RH 65%, WL at 145 ± 5 µmol m^{-2} s^{-1} intensity with R:FR ratios of 12-13 (incident light) and 2-3 (lateral at rosette stage) was used, providing a sufficiently high ratio to avoid shade avoidance syndrome (SAS) responses. Additionally, phenotypes were shuffled weekly to ensure different light conditions were distributed randomly, to exclude or minimise this factor. Water and Hyponex (pH 6.0) were given separately once a week to ensure a sufficient water- and nutrient supply so that these factors impair as little as possible.

For seedling experiments plants were grown on 1.0% DAISHIN agar (Duchefa Biochemie) with 1/2 MS (Duchefa Biochemie) for mineral supply at pH 5.8. Seeds were sterilised with 70% ethanol, 2% NaClO, rinsed 3x with ddH_2O, stratified for 2 d at 4 °C on water agar plates and cultured in climate rooms at 24 °C, in 16/8 h light cycles under WL. GR24 was dissolved in DMSO, added to the medium at 5 µmol and controls contained equivalent DMSO volumes. Etiolated plants on agar were exposed to light for 13 d after stratification and subsequently darkened for 12 d.

Treatments: 0.01% Tre (D-(+)-Trehalose dihydrate, 378.33 g mol^{-1}, Sichma-Aldrich) was sprayed (Revell Airbrush “starter class” kit) two times per week on rosettes (~0.5 ml $plant^{-1}$ $treatment^{-1}$) at the beginning of light cycles, from 25 until 49 DAS (Σ 8x). Other plants were sprayed with an equimolar sorbitol (Sor) solution 0.0048% (D-Sorbitol, 182.2 g mol^{-1}, Duchefa) at the same time to compensate for osmotic effects. After initiation of spraying, plants were only watered from the bottom to avoid a sugar residual wash off. Two extended nights (+2 h) per week were applied from 25 until 49 DAS (Σ 8x) as Tre treatments were. Luc reporter activity measurements for the confirmation of sugar starvation were taken at the end of extended nights synchronously to controls and Tre treated reporters, which then were already exposed to light for 2 h. Luciferin (1 mM) was sprayed on rosettes 30 min before photos were taken in the luminometer. The following measurements were made 24 h and 48 h later to test for sustain effects.

For the PCR analysis of the putative double mutant confirmation plants were grown on soil for 6 weeks when rosette leaf samples were taken (n>40 per genotype). These were subsequently transferred to liquid nitrogen and stored at -80 °C. Methods for DNA isolation, purification and PCR configuration can be found in the appendix.

Devices: A D5100 DSLR (Nikon, Japan) with 35 mm 1:1.8 NIKKOR prime lens (Nikon, Japan) was used for phenotype analysis and a Pixis 1024B camera system (Princeston Instruments, USA) with 35 mm 1:1.4 Nikon NIKKOR prime lens with mounted DT Green filter (to reduce auto-fluorescence chlorophyll emissions) for reporter plants. Time lapse cameras PlantCam WSCA04-00106 (Wingscape, USA) were used to track shoot development. Data logger PC sensor TEMPerHUM (RDing Technology, China) for temperature and humidity confirmation, photometer LI-1400 (LI-COR, USA) sensor quantum Q42615 (LI-COR, USA) for light quantity and R:FR meter calibrated to λ=660 nm, 730 nm (Skye, UK) for these specific wavelengths were used.

Software: For statistical analysis, RStudio 0.99, QtiPlot 0.9.8.9 and for the layout LibeOffice Calc and Writer 4.2.8.2 were used. ImageJ 1.48k for photo analysis and GIMP Image Editor 2.8 for photo processing were applied. For illustration of schemes, Gliffy online diagram and flowchart software (www.glifffy.com) was used.

Statistics: A generalized linear model (glm) with “family=Gamma” for abnormal distribution and “family=Poisson” for counts, followed by Wilcoxon Rank Sum and Signed Rank Tests (pairwise comparisons, α=0.05), Levene's Test of Equality of Variances and Shapiro-Wilk Normality Test were run with RStudio software for analysis.

Criteria and judgement: Plants were considered as bolted when the ms reached a length of ≥1 cm, while branches were counted when ≥2.0 cm. Buds were regarded as broken when outgrowth exceeded 0.3 mm. For shoot analysis plants were discarded 20 DAS when exhibiting deformity or retardation and the 15 fittest plants per phenotype were chosen. Branch and ms dry weights (DWs) included associated flowers and siliques and rosette weights included primary

rosette branches below the count threshold. Shoot architecture of *Arabidopsis* is made up of metamer unit series, which are either vegetative, consisting of a node with an axillary branch leaf, an axillary bud and an optionally elongated internode (Mündermann *et al.* 2005), or generative with a node, internode and a flower or silique. Vegetative phytomers (fig. A.1 A) of a primary cauline branch were counted by the number of visible axillary branch leaves, which bear a bud (if not already grown out) and compared to the number of countable buds or branches. Elongation of the internode and a bud break in the lateral axil, which can develop into a branch, depend on various environmental and genetic factors. In this experiment, the branching pattern of *Arabidopsis* was separated into basal primary rosette branches (<3 mm elevation on rosette) with secondary rosette branches emerging out of their axil buds in vegetative phytomers, as well as elevated cauline branches (≥3 mm elevation) with secondary cauline branches (fig. A.1 B).

Starvation measurements: Tre treated plants showed no apparent difference in morphology compared to the controls (fig. A.1 C), whereas a more slender architecture of plants under extended night conditions was noticed. To observe a sugar starvation effect of extended nights and Tre applications, starvation Luc reporter plants (fig. A.1 D-F) were grown under the same conditions as wt and *max* mutants. These were treated with the substrate luciferin to visualise starvation responses. While plants under extended night conditions exhibited a reporter activity, no meaningful difference in luciferase activity between Tre treatment and controls as well as both in extended night conditions were observed since repeated measurements of these resulted in random, inconsistent activity signals. A sustain response of extended night treatments could be ascertained up to the following day (fig. A.1 E).

Chapter 3 - Results

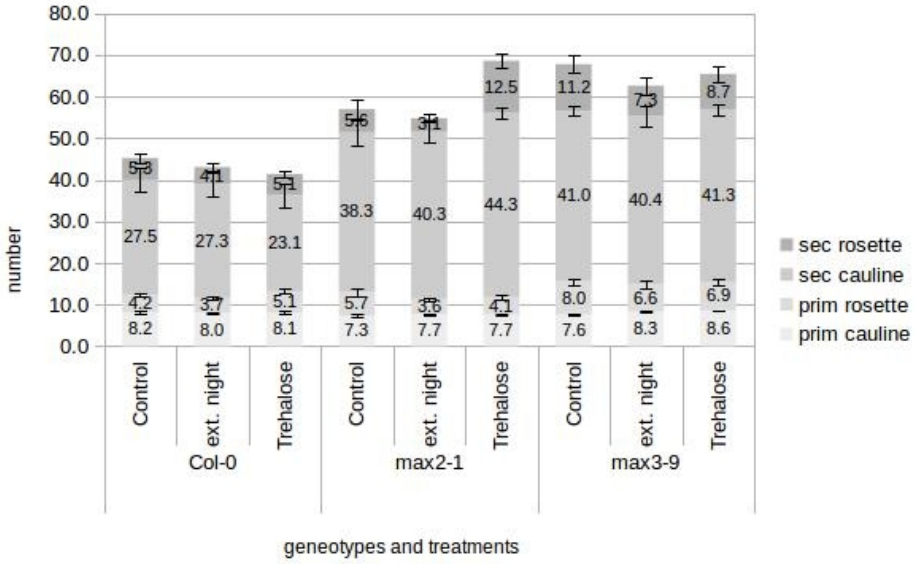

Fig. 3.1. Sum of branches per plant. Bars and labels show mean values with corresponding *SE* on top (n=15).

Tab. 3.1. Significances of pairwise comparisons of sum of branches per plant (n=15).

Treatment	Control	Control	Control	ext. night	ext. night	ext. night	Trehalose	Trehalose	Trehalose
Comparison	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*
P [a] prim. cauline						*			**
P [ab] prim. rosette		***	*		**	**		*	**
P [ab] sec. cauline	**	***		**	**		***	***	
P [ab] sec. rosette		*	*				**		
P [ab] Sum	**	***	*	*	*		***	***	
Genotype	Col-0	Col-0	Col-0	*max2-1*	*max2-1*	*max2-1*	*max3-9*	*max3-9*	*max3-9*
Comparison	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night
P [a] prim. cauline								**	
P [ab] prim. rosette						*			
P [ab] sec. cauline									
P [ab] sec. rosette				***	**				
P [ab] Sum				**	*				

[a] *Signif. codes: P <0.001 '***', <0.01 '**', <0.05 '*'.*
[b] *Signif. normally distributed.*

Sum of branches per plant

To observe the impact of altered MAX- and sugar levels on branch quantity, plants were destructively analysed by disassembling and components were photographed. No strong difference in number of primary cauline branches between genotypes, as shown by Stirnberg *et al.* (2002) and treatments were found except max mutants where Tre diminished the number in *max3-9* in comparison to the control (fig. 3.1, tab 3.1). The indifference was also true for primary rosette branch numbers between Col-0 and *max2-1* by taking the 2 cm count threshold into account, which is in contrasts with the results of Stirnberg *et al.* (2002), while *max3-9* had the highest count in all treatments (further explanations in fig. A.5). Overall, secondary cauline branches were significantly lower in Col-0 in comparison to the mutants, but not strikingly different between these (this is explained more expensively in fig. 3.2, fig. 3.3 and fig. A.6). Particularly Col-0 had a considerably lower total branch number, whereas the difference between mutants was rather weak. While extended night treatments resulted in a slightly smaller difference between genotypes in total branch number, controls and Tre treated mutants showed significantly higher variances, overall in *max3-9*. Almost no effect of treatments on number of branch types and total branch number per plant was observed. An exception was found in secondary rosette branches of *max2-1* mutants, which were sprayed with Tre and exhibited a clear increase. This treatment also contributed to a noticeable higher total branch number.

Additionally, ms lengths were analysed besides primary cauline branch count. While genotypes in all treatments differed strongly in this trait with wt displaying the longest and *max2-1* possessing the shortest ms, treatments had no significant effect on genotypes in regard to length (fig A.1, tab. A.1). However, ms weight of both mutants differed between extended night and Tre (fig. 3.5). This can either be ascribed to unequal secondary growth or a distinct difference in silique weight.

In Col-0 little branch growth took place until the growth of generative phytomers set in. max2-1 mutants exhibited slower increase in ms height and break of axillary buds on the ms started almost synchronously and slowly grew continuously before siliques were noticeable (data not shown). The outgrowth pattern of ms and primary cauline branches in *max3-9* was in between Col-0 and *max2-1*. Furthermore, no strong difference between genotypes was found in time until bolting (fig. A.3), therefore branching time was nearly equal, so this analysis was only slightly affected by developmental stage. However, a strong significant difference of extended night treatments could only be observed in *max2-1* in comparison to control and Tre application. This could indicate a higher sensitivity of these mutants to different treatments and the slightly shorter developmental time could explain a strong difference in branching traits compared to other genotypes. In general, no difference between Col-0 and *max3-9* were found in this context (fig. A.2). It is of importance to differentiate between bolting time and the floral transition, which is not synchronous (Pouteau and Albertini 2011).

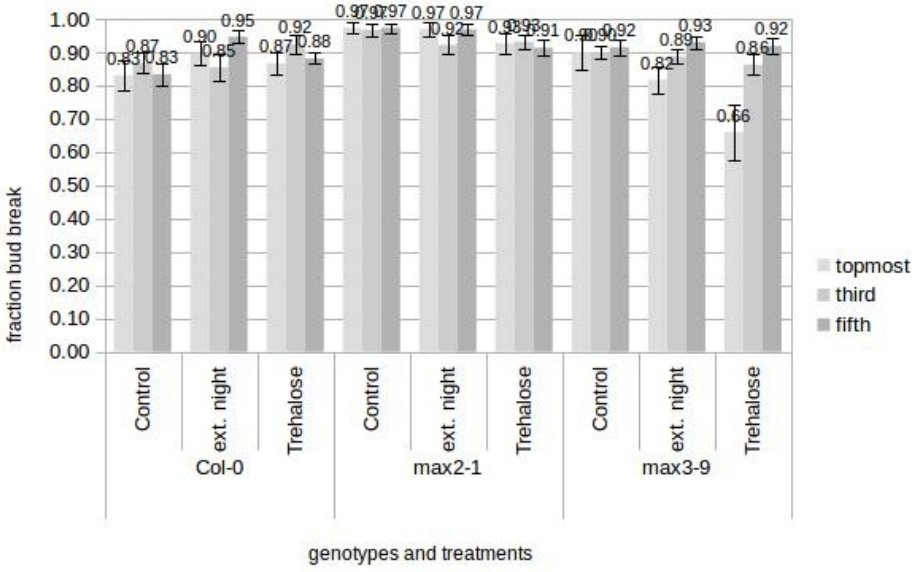

Fig. 3.2. Fraction of bud breaks on topmost (first), third and fifth cauline branches after 76 d. Count threshold for this analysis was set to 0.3 mm. Bars and labels show mean values with corresponding *SE* (n=15).

Tab. 3.2. Significances of pairwise comparisons of fraction of bud breaks (n=15).

Treatment	Control	Control	Control	ext. night	ext. night	ext. night	Trehalose	Trehalose	Trehalose
Comparison	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*
P [abc] first	*					**			**
P [ab] third	*		*						
P [ab] fifth	***		*						
Genotype	Col-0	Col-0	Col-0	*max2-1*	*max2-1*	*max2-1*	*max3-9*	*max3-9*	*max3-9*
Comparison	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night
P [ab] first								*	
P [ab] third									
P [ab] fifth	*		**		*				

[a] *Signif. codes: P <0.001 '***', <0.01 '**', <0.05 '*'.*
[b] *Signif. normally distributed.*
[c] *Signif. homogeneity of variances.*

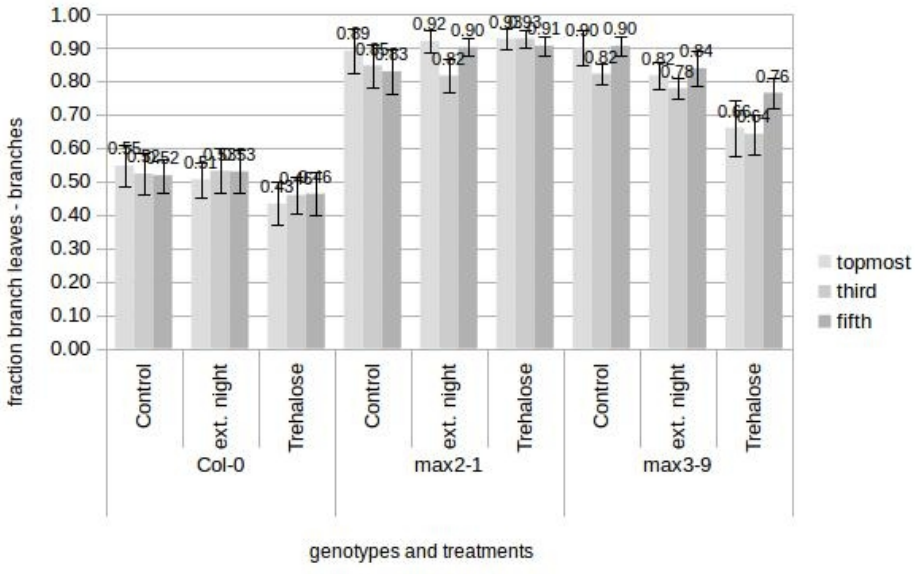

Fig. 3.3. Fractions of branch leaves to branches (>2 cm) on topmost (first), third and fifth cauline branches after 76 d. Bars and labels show mean values with corresponding *SE* (n=15).

Tab. 3.3. Significances of pairwise comparisons of fractions of branch leaves to branches (n=15).

Treatment	Control	Control	Control	ext. night	ext. night	ext. night	Trehalose	Trehalose	Trehalose
Comparison	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*
P [ab] first	***	***		***	***		***	*	**
P [ab] third	***	***		**	**		***		***
P [ab] fifth	***	***		***	***		***	**	*
Genotype	Col-0	Col-0	Col-0	*max2-1*	*max2-1*	*max2-1*	*max3-9*	*max3-9*	*max3-9*
Comparison	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night
P [ab] first								*	
P [ab] third							*	*	
P [ab] fifth								*	

[a] *Signif. codes: P <0.001 '***', <0.01 '**', <0.05 '*'.*
[b] *Signif. normally distributed.*

Fractions bud break and branch outgrowth

At this late developmental stage when rather secondary branching became a differentiating characteristic between phenotypes no considerable difference in primary cauline branch length among ranks at this developmental stage was found (data not shown). The fraction of visibly broken buds was analysed in several primary cauline branch ranks to observe possible correlative inhibition in addition. Discrimination between the developmental stages "bud break" and "branching" was considered based on thresholds in length. A general trend in bud break- or branching probability in dependence on the branch rank was not observed at this developmental stage (fig. 3.2, fig. 3.3), indicating low correlative inhibition. While more than 90% up to 97% of *max2-1* buds broke in all treatments, the count in *max3-9* was slightly lower (90-78%), except in Tre treatments, which strongly reduced this number in the topmost branch to 66% (fig. 3.2, tab. 3.2). Unexpectedly, not being in accordance with the average branch count (fig. 3.1), bud break in the wt was almost as high as in the mutants, only showing a high significance in comparison to *max2-1* under control conditions (tab. 3.2). It should be mentioned that bud break is not equivalent to branching since latter requires ongoing outgrowth as a secondary step (fig. A.17), after dormancy was overcome. Almost no considerable effect of different treatments on bud break at topmost and third rank was noticed.

In terms of branch count, a strong difference between Col-0 and both max mutants was apparent. Almost all buds in *max2-1*, which were counted as broken possessed a length of at least 2 cm. While ~90% of wt buds were regarded as broken, only ~50% were counted in this analysis. This was also constituted in fig. A.6, which displays a more even branch length distribution of secondary branch lengths in mutants. Tre treatment had a greater impact on the branch fraction in *max3-9* compared to other treatments, while this trait was insignificantly influenced by treatments apart from that. The total sum of secondary cauline and rosette branches (fig. A.6) also indicated the downside of count thresholds, neglecting those, which remain below.

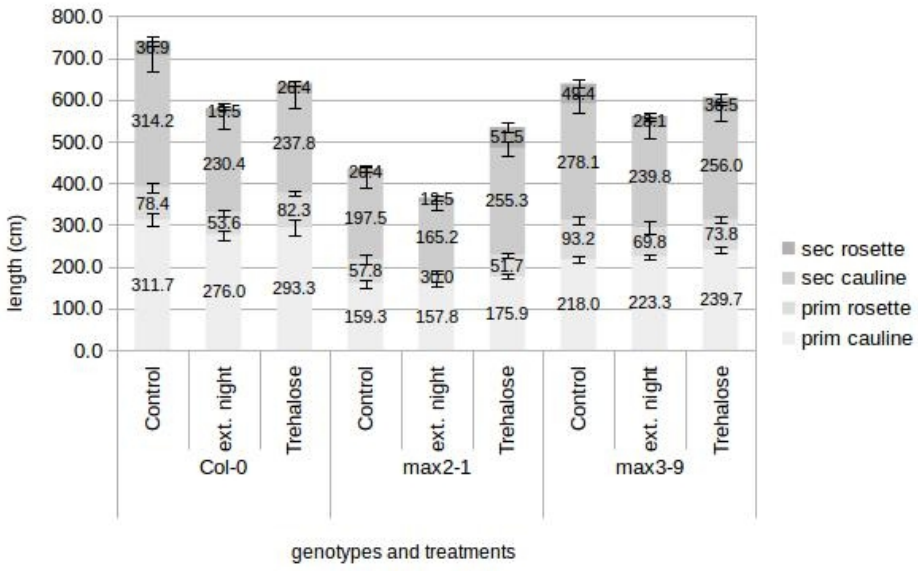

Fig. 3.4. Sum of branch lengths. Bars and labels show mean values with corresponding *SE* on top (n=15).

Tab. 3.4. Significances of pairwise comparisons of sum of branch lengths (n=15).

Treatment	Control	Control	Control	ext. night	ext. night	ext. night	Trehalose	Trehalose	Trehalose
Comparison	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*
P [abc] prim. cauline	***	***	***	***	***	***	***	**	***
P [ab] prim. rosette			**	*		**	**		*
P [ab] sec. cauline	**		*	*		*			
P [ab] sec. rosette			*				*		
P [ab] Sum	***		**	***		***	*		
Genotype	Col-0	Col-0	Col-0	*max2-1*	*max2-1*	*max2-1*	*max3-9*	*max3-9*	*max3-9*
Comparison	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night
P [abc] prim. cauline			*					*	
P [ab] prim. rosette	**			*		*			*
P [ab] sec. cauline			*	***					
P [ab] sec. rosette				***	**				
P [ab] Sum		*	***	***					

[a] *Signif. codes: P <0.001 '***', <0.01 '**', <0.05 '*'.*
[b] *Signif. normally distributed.*
[c] *Signif. homogeneity of variances.*

Sum of branch lengths

Since branch outgrowth differed from bud break characteristics, branch lengths were measured. It was not apparent if a higher branch number was linked to a greater total branch length or if outgrowth was only distributed over more branches.

Though max mutants had less cambium growth (data not shown) and a higher branch number the total branch length or sum of all branches was lower in the signalling mutant than in the wt (fig. 3.4). Col-0 and *max3-9* only differed significantly in primary cauline branch length, which was the most distinguishable trait in branch lengths, while average branch sums of *max2-1* were considerably shorter than those of wt or *max3-9*. In this regard, primary branches and ms grew slower than in Col-0 (data not shown), while more growth was distributed to secondary branches, resulting in a bushier plant. Development of total secondary branch growth was affected by genotypes and treatments to a minor extent, but this could be noticed in single branch lengths (fig. A.6). A strong difference between max mutants in control and extended night treatment was observed, but not in Tre applications. *max2-1* mutants responded more sensitive to different treatments, while was not applicable to *max3-9* (tab. 3.4). Only in *max2-1* a branch growth enhancement in Tre treatment could be found, while this was slightly diminished in growth of other genotypes. In conjunction with ms length, primary cauline branches grew to a similar extent (data not shown), indicating a conserved allometric pattern in all genotypes with a high correlation (R^2>0.8). A strong reduction of AUX synthesis in the SAM can be observed after the transition to florescence (Prusinkiewicz *et al.* 2009), resulting in a bolting response by facilitating internode elongation of phytomers below the topmost (apical) meristem. When a high number of cauline buds is broken at the same time when apical dominance in a branch is lowered, it indicates lower correlative inhibition in max mutants or a high PIN concentration on PMs for export. Since these branches have an equal potential to the ms in development (Prusinkiewicz *et al.* 2009), an inhibition or outgrowth of secondary branches could be judged equally. Between bud break and “coflorescence” (Mündermann *et al.* 2005) a high AUX export proceeds, supplying lower phytomers in the branch at high concentrations. Since total length of secondary branches in the wt did not differ strongly from mutants unlike branch numbers, a higher PIN concentration in max mutants did not remarkably contribute to a total outcome in length distribution. This is also shown in fig. A.6.

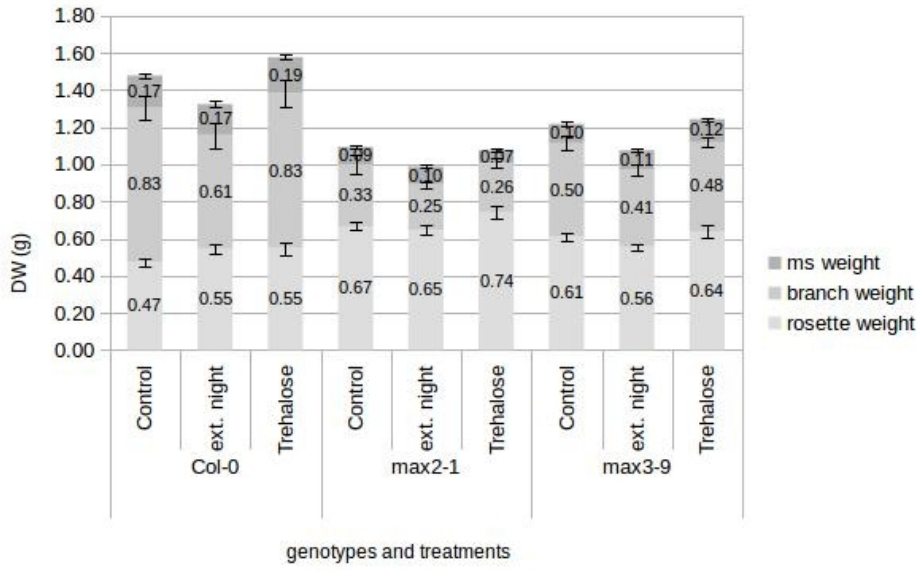

Fig. 3.5. Total shoot DW. Bars and labels show mean values with corresponding *SE* on top (n=10).

Tab. 3.5. Significances of pairwise comparisons of total shoot DW (n=10).

Treatment	Control	Control	Control	ext. night	ext. night	ext. night	Trehalose	Trehalose	Trehalose
Comparison	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*
P [a] rosette	***	***		**		**	**		*
P [ab] branches	***	***	**	***	*	***	***	***	***
P [a] ms	***	**		**	*		***	**	***
P [ab] Sum	*	*		**	*		***	**	
Genotype	Col-0	Col-0	Col-0	*max2-1*	*max2-1*	*max2-1*	*max3-9*	*max3-9*	*max3-9*
Comparison	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night
P [a] rosette				*					*
P [ab] branches	*		*						
P [a] ms				*			*		
P [ab] Sum	*								

[a] *Signif. codes: P <0.001 '***', <0.01 '**', <0.05 '*'.*
[b] *Signif. normally distributed.*

Shoot weight

Based on the observation of a higher branch count yet lower total branch length in max mutants dry weight (DW) measurements of shoot components were carried out. According to the sum of branch lengths *max2-1* had the lowest DW, while the wt showed the highest. Branch DW was the most differing trait in this context. No differences between control and Tre treatment were present, while a slightly greater effect was observed in the comparison of extended night and Tre treatment. A noticeably, but insignificantly lower total shoot weight in all genotypes treated with extended nights was found despite a total delay of only 16 h light, indicating a lower photosynthetic performance (carbon acquisition) or a higher respiration under this condition. On the other hand, Tre application did not affect the total shoot DW. Mutants and wt differed strongly in almost all shoot DW categories (tab. 3.5). Tre only increased rosette DW in *max2-1* significantly and also lowered the ms DW (fig. 3.5, tab 3.5). A conspicuously different biomass allocation within the rosette is given in fig. 3.5. In the wt only a third of shoot DW was located in the rosette, while *max2-1* allocates two thirds and *max3-9* half of its shoot biomass in this shoot component. This pattern is not in accordance with the measured projected rosette area and seems to display a high difference in SLA at first glance (more on this in fig. 3.6, fig. A.4, fig A.4). DW measurements of the ms were prone to higher relative measurement errors due to low weight, so judgement of this value is precarious. Only in Tre treatment a significant difference between *max2-1* and *max3-9* in ms DW was found with a clearly higher weight in latter. Total shoot DW was almost not affected by different treatments, rather supposing an effect on allocation within the shoot.

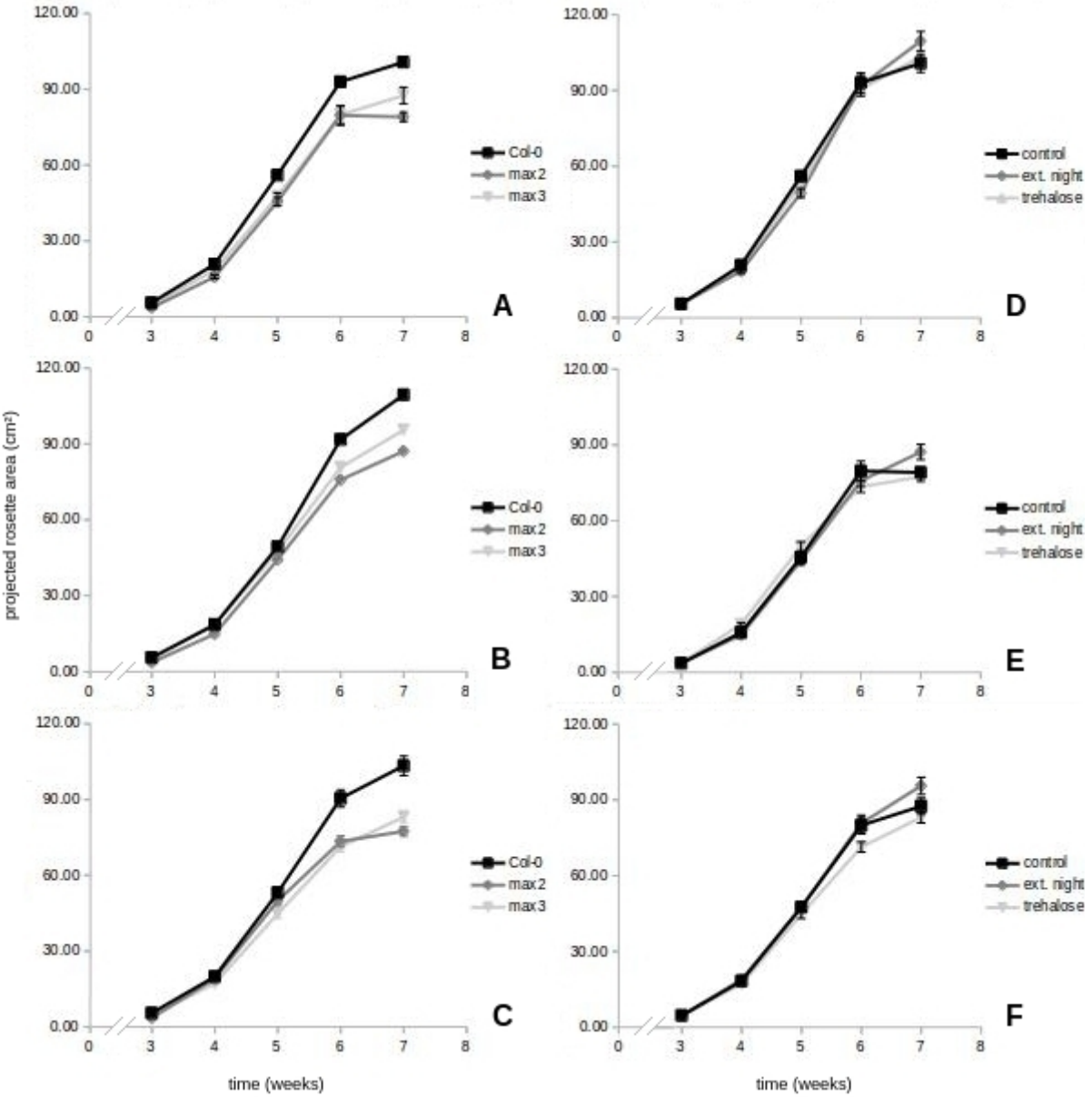

Fig. 3.6. Projected rosette area development from 21 d until 49 d of different genotypes and treatments. (A-C) Treatments of genotypes. (A) control, (B) extended night and (C) Tre application. (D-F) Genotypes in different treatments. (D) Col-0, (E) *max2-1* and (F) *max3-9*. Data points show mean values with corresponding *SE* (n=15).

Projected rosette area

Since it was presumed to obtain a higher total branch length (fig. 3.4) in accordance to the branch number (fig. 3.1), measurements of the projected rosette area as an indicator for source strength were carried out. As the major source of photosynthates, thus considerably affecting the branching progress, development of rosettes areas was tracked weekly until day 49 (fig. 3.6), when plants started shifting to the generative stage almost synchronously (fig. A.3, tab. A.2). The increase of projected area size closely followed a sigmoid curve (Boltzmann fit) with little deviation in all genotypes and treatments (fig. 3.6, tab. 3.7). When bolting was initiated, rosette growth almost reached its maximum diameter (data not shown). Col-0 plants possessed “lanceolate” (Eveland and Jackson 2011) leaves, an open rosette with radial shape, the largest projection area in all treatments (fig. 3.6) and low self shading (fig. A.4). *max2-1* mutants showed the smallest rosettes, almost insignificantly smaller than *max3-9* (tab. 3.6), the shortest petioles, though these were the longest at seedling stage (fig. A.15, tab. A.6), a closed rosette with high self-shading and wide, uncoiled leaves, which started yellowing slightly earlier than in other genotypes (data not shown). Rosettes of *max3-9* were marginally rounder, though having a rather radial shape and more overlapping than in Col-0 rosettes was identified (fig. A.4).

Tre did not substantially shift the maximum RGR of projected leaf areas (inflection point x_0), but extended night treatment did, which indicated a general delay in developmental time (tab. 3.7). In sum, 16 h of illumination were

missing, which is equal to 0.2 weeks developmental time and approximately corresponded to the calculated shift of x_0. Additionally, increase in projected rosette area development levelled off to a smaller extent under extended night conditions in comparison with control and Tre (fig. 3.6. B) after week 6, indicating a developmental delay. The *max2-1* mutant exhibited a higher sensitivity to different treatments and featured the smallest final rosette size. Tre application marginally resulted in the smallest rosette size in the mutants, whereas final size of Col-0 was insignificantly larger than under control conditions (fig. 3.6, tab. 3.6). Inner leaves of rosettes were not smaller in extended night treated plants (data not shown), which would have explained a larger leaf surface at an expense of younger leaves in the rosette. In all genotypes extended night generated the largest projected rosette area after 6 weeks and the Col-0 genotype, which was not significantly affected by different treatments, had constantly the biggest at all times and seemed to be most susceptible to treatments in this aspect (tab. 3.6). A measured decrease in *max2-1* mutants under control conditions after week 6 is ascribed to down bending leaves (fig. 3.6 A, E). Though the projected rosette area was smaller in the mutants, overall *max2-1* displayed a bushier, higher, bulkier rosette, which implies a surface increase that was not captured in these measurements, such as a halved sphere, which possesses twice the surface area ($½\ 4\ r^2\ \pi$) of a circle ($r^2\ \pi$) with equal diameter. Since the initial value has to be 0 at 0 DAS, this fit function does not truly describe the growth pattern from the beginning, but captures landmarks of the major rosette size stages. Summarising, max mutants had insignificant differences at week 7 and Col-0 started to differentiate in projected rosette area after week 6 under different treatments, but not significantly as well.

Tab. 3.6. Significances of pairwise comparisons of projected rosette area (n=15).

Treatment	Control	Control	Control	ext. night	ext. night	ext. night	Trehalose	Trehalose	Trehalose
Comparison	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*
P [ac] 21 d	***		*	***	**	*	***	**	*
P [a] 28 d	**			**		**			
P [a] 35 d	***	***						**	
P [ab] 42 d	**	**		**			***	***	**
P [ac] 49 d	***	*		***	*		***	***	
Genotype	Col-0	Col-0	Col-0	*max2-1*	*max2-1*	*max2-1*	*max3-9*	*max3-9*	*max3-9*
Comparison	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night
P [ac] 21 d									
P [a] 28 d				***	**				
P [a] 35 d				*					
P [ab] 42 d							**	*	
P [ac] 49 d				**		**	**		

[a] *Signif. codes: P <0.001 '***', <0.01 '**', <0.05 '*'.*
[b] *Signif. normally distributed.*
[c] *Signif. homogeneity of variances.*

Tab. 3.7. Boltzmann fit function of projected rosette areas mean values from 21 d until 49 d (n=15).

Genotype	Treatment	Initial value (cm^2) [a]	Final value (cm^2)	Inflection point (x_0, weeks)
Col-0	Control	0.72 ± 1.38	105.89 ± 4.30	4.92 ± 0.06
	ext. night	0.63 ± 1.10	119.71 ± 5.40	5.25 ± 0.08
	Trehalose	0.47 ± 0.96	109.96 ± 3.40	5.04 ± 0.05
max2-1	Control	0.45 ± 1.59	81.88 ± 4.48	4.85 ± 0.11
	ext. night	0.17 ± 0.23	91.36 ± 1.11	5.04 ± 0.02
	Trehalose	-1.19 ± 0.89	79.58 ± 1.82	4.66 ± 0.04
max3-9	Control	-0.29 ± 1.65	93.22 ± 4.83	4.95 ± 0.08
	ext. night	-1.97 ± 1.09	104.59 ± 3.42	5.10 ± 0.05
	Trehalose	-0.26 ± 0.03	87.45 ± 0.09	4.96 ± 0.00

[a] Initial and final values indicate the lower and upper asymptotes of the Boltzmann fit function.

Chapter 4 – Discussion

Respectively the initial intention to observe the impact of altered SL and sugar metabolism, a higher branch count in both mutants but less total branch growth in *max2-1* could be observed in both mutants in comparison to the wt. Speculation of an deviating branching result between max mutants due to mutations at different SL pathway stages could be affirmed. While *max3-9* produced more branches than *max2-1* in control and extended night treatments, Tre applications showed an opposite effect. The wt and *max3-9* exhibited the largest sum of branch lengths, while *max2-1* possessed the shortest. Under the applications of the mentioned counting method, extended nights had a negligible diminishing effect on bud break in all genotypes, while Tre slightly boosted branch numbers in *max2-1*. Both treatments showed a similar impact on total branch length, which is associated with branch numbers. In this aspect branch lengths of the Col-0 wt were reduced by both sugar alterations to a significant extent.

Branch number and outgrowth

Besides higher branch numbers (fig. 3.1, tab. 3.1), a shorter ms (fig. A.2, tab A.1) (Stirnberg *et al.* 2002) and less secondary growth by comparing ms and branch lengths with their weights (Brewer et al. 2013) in mutants were found. Furthermore it was approved that in Columbia wt all cauline buds break under favourable conditions, whereas only a small number of rosette buds do so (Finlayson *et al.* 2010) and axillary shoots of max mutants retained high activity, being richer in secondary branches compared to the wt (Stirnberg *et al.* 2002). Number of rosette branches of Col-0 and *max2-1* did not differ significantly, according to measurements with 2 cm length threshold, but since branch leaves in the *max2-1* rosette (fig. A.5) were present in high numbers, this count methodology cannot be assured. Because the number of primary cauline branches was negligibly affected by genotype and treatment, no internode elongation of further phytomers in the rosette proceeded, supposing this growth pattern to be a conserved or unimpaired by these metabolic alterations. A slower ms elongation (cm d^{-1}) in plants of 10 h compared to 12 h DL was observed by Pouteau and Albertini (2011)**,** which was also measured in ms of intermittent extended night treated plants (data not shown), but these resulted in a shorter final length and validate the growth inhibiting effect of this treatment. In contrast to observations of Mündermann *et al.* (2005), a higher number in secondary branches was observed in both mutants compared to the wt when the branch threshold is taken into account (fig. 3.1, tab. 3.1, fig. A.6). This may also be dependant on the developmental stage (fig. 3.2, tab. 3.2, fig. 3.3, tab. 3.3).

It was proven that Suc has a promoting effect on bud outgrowth (Mason *et al.* 2014). Although Tre might have enhanced a bud break response by its precursor T6P (Yadav *et al.* 2014, Schluepmann and Paul 2009) a higher availability of Suc was thereby not given and since cell elongation is the growth key factor for those cells, which are no longer in the meristem position and eventually differentiate (Francis and Halford 2006, Wang and Ruan 2013). A more even branch length of second degree also originated from an earlier growth initiation (data not shown), which could have proceeded at a higher paste at the beginning (Stirnberg *et al.* 2002). This incidence also provides an indication of very low correlative inhibition in the max mutants compared to the wt and could also be explained by altered biomass allocation to axillary growth since primary branches were shorter though.

Shoot DW and carbon allocation

A higher shoot weight in the wt indicated higher carbon acquisition or lower respiration than in the mutants. These rather exhibited a high carbon investment in the rosette instead of elevated components, resulting in a clearly different biomass ratio of shoot components. According to the trophic hypothesis (Rameau *et al.* 2015), bud outgrowth is associated with a fractionation of starch in stem tissues and higher enzymatic activity in sugar metabolism. Tre application indirectly mimics sugar abundance, but can also disturb the balance of starch metabolism at high concentrations. In the same context, CKs are also involved in nutrient remobilization towards sinks (Yamada and Umehara 2015) and are affected by the concentration of SLs, according to the second messenger hypothesis. Though it could be confirmed that the wt had the strongest secondary growth (Agusti *et al.* 2011), weight of the branches and attachments lead to a bend over of the shoot 70 DAS as in the mutants with higher numbers in branches, shorter stems and less secondary growth (data not shown). It is uncertain if Tre increased weight of siliques (Stoller *et al.* 2012) since these were not weighed separately. Siliques as indicators for generative metamer numbers at the apex ends of primary branches were were negligibly diminished in *max3-9*, while *max2-1* mutants possessed less than half of those of Col-0 at 76 d (data not given). This difference in generative metamers indicated a great difference between the mutants, but is also closely connected to developmental time. In this regard, the approach to breed a crop, in which SL synthesis is excluded or suppressed in order to prevent an attraction and infestation by SL responding parasites, such as *Striga*, would very likely result in a lower (fruit) yielding cultivar.

Rosette development and characteristics

The strong difference between genotypes in rosette morphology gave occasion to consider this shoot component as well. A pleiotropic effect of max2 mutation (Stirnberg *et al.* 2002, Shen *et al.* 2007, Tsuchiya and McCourt 2009) could

likewise be observed in this trait. However, an increase in leaf size can have several backgrounds, such as interference in the GA metabolism (Paparelli *et al.* 2013). The actual leaf area could not be measured with this method but provided a rough indication of light capturing and is nondestructive. As mentioned, the rosette height also had an impact on surface area and is a strong distortion factor when individuals differ in this characteristic. The abundance in leaves of mutants was shown in isolated rosettes (fig. A.5), which presumably had a substantial effect on rosette mass (fig. 3.5). However, approaches of measuring the exact leaf area became apparent to be elaborate and laborious. Supporting the sink theory bud break can also be triggered through the removal of eutrophic, outgrowing (cauline) leaves (Mason *et al.* 2014). Given observations support the sink theory by enhanced rosette leaf growth in conjunction with lower branching.

While all primary cauline buds in Col-0 broke and developed into branches this is not the case for lower buds in the rosette (Finlayson *et al.* 2010), which could be confirmed. A higher number of leaves located in the rosette could be observed in both mutants (fig. A.5), which either indicates a higher number of lower phytomers in comparison to the wt or an advanced phytomer development, at which the branch leaf, as a branch precursor (Pouteau and Albertini 2011) is already visible. The group around Finlayson also showed the number of primary rosette buds of *max2* mutants were almost the same as in the wt. Since MAX2 is essential in each axillary bud to inhibit growth (Stirnberg *et al.* 2007), it rather brings the fate of bud outgrowth into focus than a difference in bud number and therefore a similar branching opportunity is given.

Another aspect would be the “cost-benefit analyses” as described by Coste *et al.* (2011). The lower rosette leaves are generally the oldest, increasingly larger ones (Stirnberg *et al.* 2002), which receive less sunlight at later rosette stages due to self-shading from newer leaves on top. A replacement of older leaves by newer ones is not always associated with leaf shedding. The investment in new petiole and leaf lamina tissue was described to be in conjunction with the “specific construction cost” and “dimensional properties” (Coste *et al.* 2011). Due to the rosette architecture, an excess of leaf production with equal lengths would rather increase the maintenance costs due to self-shading since the “lanceolate” leaf shape and “golden angle” phyllotaxis of the wt already captures radiance optimally. Therefore, additionally developed rosette leaves in *max2-1* and *max3-9* (fig. 3.5, fig. A.5) rather act as carbon sinks for maintenance and become carbon source competitors for the rest of the shoot. Young rosette branch leaves (fig. A.5) probably could not reach their turnover rate or “payback time for leaf area deployment” (Coste *et al.* 2011) and therefore rather take the role of competitive sugar sinks for branches and also presumably withdraw photosynthetic proteins from older leaves. Since AUX is transported basipetally, these young leaves as possible AUX sources (Ljung *et al.* 2001) probably cannot contribute to branching enhancement, but rather to root growth. The shift of biomass allocation to the rosette is a strong, but not a valid argument for the trophic hypothesis, in respect to lower branch DW, making argumentation susceptible to “affirming the consequent”.

Disconfirmation of the putative *max2/max3* double mutant

Initially, a branching analysis of a cross between *max2-1* and *max3-9* mutants was designed. Phenotypes of this putative double mutant, which shared characteristics of both parental lines were selected and F4 generation plants were grown. Anyhow, PCR results of these confirmed the absence of the *max3-9* fragment (fig. A.7), so these genotypes were excluded from the experimental setup. *max3-9* mutants originated from a Landsberg *erecta* (L*er*) background and were backcrossed with Col-0 over eight generations. These could still show pleiotropic effects, known for *erecta* plants, such as affected GA- and AUX metabolism, responses to circadian cycle, or a different hypocotyl or petal length (Zanten *et al.* 2009, Abraham *et al.* 2013).

Besides branch and rosette analysis, other aspects were observed in order to gain deeper insight in the effect of SL mutations and sugar treatments.

Senescence

Since no delayed but a marginally premature senescence reaction could be observed in mature rosette leaves of mutants relative to the wt (data not shown), the experiment of Woo *et al.* (2001) and Yan *et al.* (2007), focussing on leaf senescence was similarly repeated with leaves of *Arabidopsis* in regard to the MAX2 ortholog D3 (Leyser 2008, Yamada and Umehara 2015), which is part of the senescence pathway. Contrary to MAX2, CKs inhibit leaf senescence. (Yamada and Umehara 2015). It could be confirmed that the senescence progression of *max2-1* leaves is delayed or decelerated, while those of Col-0 and *max3-9* showed a similar decay response (fig. A.8, fig. A.9, fig. A.10, fig. A.11). It is assumed leaves are shed in dependence of their life span and carbon balance, as soon as younger leaves possess a higher carbon turnover (Coste *et al.* 2011). Shedding of *max2-1* rosette leaves despite earlier yellowing at the edges could not be observed. Though chlorophyll content was not measured, the product of HSV saturation and RGB green value over time provided information on this progress (fig. A.9, fig. A.10, fig. A.11).

To observe if this progression can be found in attached leaves as well plants were grown on agar, illuminated for 13 d and subsequently etiolated for 12 d (fig. A.12). Unlike in isolated leaves, all genotypes showed a senescence response, which indicates either a partial retraction of chlorophyll, or initiated autophagy to serve as energy supply. This was in accordance with the change in colour of shaded rosette leaves in *max2-1* and *max3-9* mutants after ~60 d,

whereas this response could be raised by leaf life span as well (Coste *et al.* 2011). A delayed senescence induction in *max2* (Yan *et al.* 2007) could therefore not be observed, but leaves could also not be assessed as dead since this response was not found in the entire leaf.

Seedling experiments

To observe whether embryonic organs in these genotypes already differ, both hypocotyls and petioles were observed. The max2-1 mutant possesses ~60% more hypocotyl epidermis cells, while there was no relevant difference in cell number between Col-0 and *max3-9* (fig. A.13, tab A.4), which indicated a remarkable difference between the mutants. It has been asserted that hypocotyl growth neither includes cell divisions in cortical nor in epidermal cells to a significant extent (Gendreau *et al.* 1997), which explains the indifference between control (DMSO) and GR24 treatment and impedes the analysis of GR24 effects.

In terms of hypocotyl length, GR24 application had an strong impact in all genotypes, with a slightly weaker response to GR24 in *max2-1*, whereas Col-0 and *max3-9* rather showed similar responses in both treatments (fig. A.14, tab. A.5). A fraction of hypocotyl length and hypocotyl epidermis cell number also proved epidermis cells of *max2-1* not only being in higher numbers, but also longer on average (fig. A.13, fig. A.14). It could be confirmed that SLs have an inhibiting effect on hypocotyl length and that *max2-1* had the greatest length (Stirnberg *et al.* 2002, Tsuchiya *et al.* 2010, Wang *et al.* 2013), which could be explained by higher AUX concentrations in max mutants (Agusti *et al.* 2011). However, *max3-9* seedlings neither showed a difference to the wt in cell number nor hypocotyl length. Petiole length of Col-0 and *max3-9* were almost equal under control conditions (fig. A.15, tab. A.6), but showed different responses to GR24, to which *max3-9* mutants responded with a 55% reduction. This would be indicative of the compensating up-regulation through AUX (Mashiguchi *et al.* 2009) and according to the SL pathway of Yamada and Umehara (2015) the presence of SL would skip the *MAX3* transcription step, compensating or replacing the missing component (MAX3) so no difference between wt and *max3* in GR24 treatments should have been observed. It could be approved that *max2* had longer petioles (fig. A.15) than the wt (Shen *et al.* 2007) and *max3* in cotyledons and in contrast the shortest in mature leaves (Stirnberg *et al.* 2002). The weakest effect was observed in these with a 7% decrease, which was still strongly significant (tab. A.6). Since transport of GR24 or SLs from growth medium to the apex is considerably shorter during seedling stage than in mature plants, the transfer pattern might be different. In contrast to the hypocotyl, cells of the epigeal cotyledons in Arabidopsis are subjected to cell divisions during their outgrowth (Stoynova-Bakalova *et al.* 2003), but those were not quantified in this analysis, so no clear statement can be made if GR24 acts as a cell division or expansion inhibitor in this organ.

Though experiments focused on shoot development, the effect of GR24 on the primary root length was observed as well. Contrary to the relative response of max mutants on GR24 in petiole length, a remarkable growth inhibition (79%) was found in *max3-9*, which had a similar length to the wt in controls (fig. A.16, tab. A.7). Effects of SLs on PIN1 on the PM in roots still demand further research (Shinohara *et al.* 2013). Under given conditions, primary root length in wt was the longest in both treatments and *max2-1* was inhibited by GR24 almost to the same extent in contrast to results of Shinohara *et al.* (2013).

Tre and Sor applications

According to a remarkable effect on crop yield increase (Stoller *et al.* 2012) and improved resistance against biotic stress by indirectly strengthening epidemal cell walls (Reignault *et al.* 2001), quantities already differed immensely from each other if applied to *Arabidopsis* (≈123 µg and ≈15000 µg Tre plant^{-1}). Thus, a proper reference for the application with focus on branching was not given. Since plants were sprayed two times per week for 4 weeks, a high concentration as from Reignault (Reignault *et al.* 2001) would probably have caused a thick sugar coating. Since no high quantities of endogenous Tre or T6P are present (Paul *et al.* 2008), the low employed concentrations (≈400 µg Tre *Arabidopsis*$^{-1}$) could be justified. In consequence of using only one Tre concentration, results of various levels are missing and cannot provide information to a vast extent. Though Sor, as an osmotic compensator for Tre treatment has almost no effect on bud growth (Mason *et al.* 2014) it might have affected other processes, which could influence the overall growth response. Tre application did not induce an unambiguous response in the starvation Luc reporter plants in both extended nights and constant day length (DL) (fig. A.1 D-F), though spraying Tre and thereby establishing higher levels of the precursor T6P (chemical equilibrium) should have implied a strong inhibition of starch degradation (Martins *et al.* 2013). If Tre was distributed over the whole organism and T6P limited to the cell level as suggested by Schluepmann and Paul (2009), a sugar surplus signalling could have been bound to the rosette but not conveyed to other parts of the shoot.

Glucose (Glc) is involved in the entire cell cycle mechanism, including mitotic frequency, primarily depending on Glc signalling in combination with hexokinase (HXK), which is mainly participating in gene expression (Wang and Ruan 2013). It triggers the shift from gap phase 2 (G2) to mitosis phase (M) inhibiting the suppressors of this step and contributing to the transcription of the necessary molecules (Skylar *et al.* 2011). Besides Glc, AUX is also required for cell division, suggesting an interaction between both factors in the shift to M (Skylar *et al.* 2011). Suc also inhibits the

synthesis of BRANCHED1 protein (BRC1), which suppresses bud break (Mason *et al.* 2014). Since Suc and Glc are missing despite sugar excess signalling through T6P, a weak effect of Tre spraying could be elucidated.

Besides quantity, the start of application could have been set earlier at vegetative stage since a higher susceptibility to treatments could be expected. Due to low TreH activity, application of Tre on seedlings results in a negative development besides inhibition of starch degradation (Schluepmann and Paul 2009), wherefore Tre application was initiated at a later stage, when plants had approximately 10 leaves. Suc availability in combination with T6P supports seedling growth, while a deficiency and yet high levels of Tre and thus T6P have a negative effect (Schluepmann *et al.* 2004). Later in vegetative stages, higher concentrations increase rubisco and chlorophyll and therefore boost the photosynthetic capacity (Schluepmann *et al.* 2004, Schluepmann and Paul 2009) and its levels rise after Suc addition (Yadav *et al.* 2014). A strong enhancing effect in form of bigger branch lengths or higher biomass could hardly be observed. Besides this, the degree of Tre uptake through the adaxial epidermis is unknown.

Extended nights

Under given conditions a sustained effect exceeding one day could be observed in extended night treatments, while starvation Luc activity was not stimulated in Tre application (fig. A.1 D). Determining a proper dosage was challenging for both treatments. Too high frequencies of night extensions would have led to a short day (SD) adaptation of the plants and an even stronger shift of the maximal RGR in projected rosette area, while a lower frequency probably would have had almost no effect on development. Since starch degradation takes place in an almost linear manner (Graf *et al.* 2010) until almost depleted (Martins *et al.* 2013) to facilitate energy for respiration (Izumi *et al.* 2013, Martins *et al.* 2013), shorter sugars are depleting quickly within the first hours of darkness followed by a marginal decrease. In these conditions plants react to this by subsequently adapting their metabolism, which can involve a memory effect (Cordoba *et al.* 2014), that was displayed in starvation Luc reporter plants (fig. A.1). By covering plants to extend the night cycle, a negligible temperature increase was measured inside (+0.2 °C), which probably enhanced starvation due to increased respiration. Whether the branching results of extended nights is caused by reduced sugar signalling or a general deceleration of development is unclear (fig. A.17). A shift of the inflection point (highest RGR) was almost linear to a reduction of developmental days. *Arabidopsis* is known to be affected by a reduction of DL in shoot architecture and generative traits (Beveridge *et al.* 2003) by increasing the rate of starch synthesis to facilitate an adequate starch synthesis to adapt to longer nights (Martins *et al.* 2013). Though sugar starvation is linked to senescence associated genes (Lee *et al.* 2004), no noticeable effect could be observed.

SnRK1-like proteins are involved in the regulation of energy balance and stress signalling (fig. A.17) and the highest transcription is found at insufficient starch levels. These kinases are inhibited by T6P (Smeekens *et al.* 2009, Arias *et al.* 2014) and their subunits SNF1-related protein kinase catalytic subunit alpha KIN10 (KIN10) and KIN11 are present in *Arabidopsis* (Cordoba *et al.* 2014). Thus, it might be possible Tre applications suppressed a starvation response. GA synthesis is dependent on the daily photosynthetic performance, implementing a growth pattern, which is matching to the environmental conditions (Paparelli *et al.* 2013). This hormone is of importance in internode elongation (Rameau *et al.* 2015), so reduced branch outgrowth can also be attributed to lower GA levels (Paparelli *et al.* 2013).

Set up

A slender plant with little lateral growth is rather able to out-compete its neighbours according to light capturing (Brewer *et al.* 2013). In most plants low R:FR ratios decrease the extent of branching, which is a result of phytochrome B (PHYB) inactivity (Leduc *et al.* 2014). *MAX2* and *MAX4* are essential genes for PHYB to stimulate bud initiation and outgrowth and diminishing correlative inhibition (Leduc *et al.* 2014), so it is assumed that MAX2 supports the inhibition of bud outgrowth in dependence on light environments (Leduc *et al.* 2014) and PHYB positively affects BRC1 transcription under low R:FR conditions (González-Grandío *et al.* 2013). Besides this, the *PHYTOCHROME-INTERACTING FACTOR (PIF*) family diminishes auxin synthesis when initiated by Glc (Wang and Ruan 2013). R:FR ratios of incident and lateral light until the end of rosette stage were sufficiently high to avoid shade avoidance syndrome (SAS) responses. It is assumed that buds possess all groups of photoreceptor (Leduc *et al.* 2014) and phytochrome genes are basically expressed in all plant organs in dependence on the growth progress (Leduc *et al.* 2014).

It has been proven that SLs production and auxin signalling (Koltai 2012, Brewer *et al.* 2013) is boosted in roots of inorganic phosphate (P_i) lacking plants (López-Ráez *et al.* 2008), including *Arabidopsis* (Hammond and White 2011), resulting in reduced shoot branching (Kohlen *et al.* 2011). For this reason, plants were regularly supplied with Hyponex in order to prevent this response and to avoid growth limitation due to nutrient/mineral starvation.

Critiques of methods and analysis

Branches were counted when their length was ≥2 cm according to Stirnberg *et al.* (2007). Distortions in counts by applying this threshold could be noticed in branch numbers of rosette branches (tab. 3.1), which indicated presence of a high primary rosette branch leaf number in mutants (tab. A.4) but only in *max3-9* mutants a noticeable number crossed

the 2 cm count threshold. Thus, an insignificant difference in primary rosette branch counts between Col-0 and *max2-1* (tab. 3.1) was achieved and cannot disprove results of publications claiming a remarkable increase of these branches in *max2-1*. Though differences between projected rosette area size in Col-0 under different treatments was not significant ($P>0.05$), a trend was still noticeable. Anyhow, if a consequence is still relevant, it can be taken into consideration nonetheless. Therefore, a differentiation between relevance and significance is of importance (Arens *et al.* 2008, Leek and Peng 2015). Taking all plants into account for analysis, which were regarded as healthy and were chosen 19 DAS ensured a balanced design for statistics and revealed a more representative sample group. This also implied a high, inhomogeneous variance due to stunted individuals (outliers). That type of approach should defy a confirmation bias, to which analysis can be prone due to expectations.

Future experiments

Although experiments with SL signalling-synthesis double mutants on branching have already been conducted (Stirnberg *et al.* 2002), analyses with mutants featuring the *MAX2-1* and *MAX3-9* fragments should be carried out to find further explanations of whether there is an antagonistic, additive, synergistic or cancelling effect of this combination on branching architecture. If local expression of BRC1 is actually terminated as soon as an axillary bud breaks, molecular BRC1 analysis could reveal if buds can directly be broken by Tre application in the wt and if BRC1 is expressed at a detectable intensity in max mutants, since our results rather indicate a difference in biomass allocation for outgrowth than bud inhibition. In this context concentrations of BRC2, which is involved in branch outgrowth (Finlayson *et al.* 2010) should be compared between genotypes. Furthermore, quantities of Tre application could be increased in future experiments, since it was calibrated closely to the dosage of Stoller *et al.* (2013) on wheat crops (~3x lower) and showed a noticeable but weak effect on branching. On the other hand, applied quantities may have been too high and had an inhibiting effect. It was also not observed if outgrowth of additional, young rosette branch leaves set in before or after bolting and if rosette weights decrease, increase or do not alter after inflorescence. Since extended nights have a more extensive effect than just sugar signalling (fig. A.17), another methodology needs to be utilised. Further research on MAX should elucidate how it affects the Cyc synthesis, degradation, activation and inhibition, since these play a crucial role in the cell cycle.

Conclusions

Luminometer photos displayed a starvation Luc reporter response after extended nights with a sustain up to one day, while effects of Tre application were not noticeable. Both treatments had almost no effect on branch number and therefore bud break. A higher susceptibility to extended nights was found in *max2-1*, which indicates MAX2 signalling to buffer against changes in shoot development in this condition. Since both sugar treatments affected secondary branch growth to a higher extent than primary, a developmental delay or weakened shoot outgrowth could be assumed, also referring to slightly lowered branch weight under extended nights. Therefore, the assumption extended nights diminish the branching could be approved to a certain extent. However, Tre treatment marginally increased rosette mass, which could confirm the inhibition of sugar export due to high T6P concentrations since total shoot DW was not affected. Thought these treatments had almost no effect on projected rosette area, plants had a slightly bigger projected rosette area under intermittent extended night conditions.

Apart from boosted axillary bud break, total branch outgrowth in max mutants was diminished due to a reduced internode elongation. Nevertheless, primary cauline branch numbers were almost unaltered, thus no internode elongation of additional phytomers in the rosette was present, ascribing a primary branching potential to the rosette and indicating a conserved or unaffected ms configuration. The higher similarity in traits of *max3-9* to the wt in comparison to the signalling mutant provide an indication of the leakiness theory of SL synthesis mutants (Cheng *et al.* 2013) or a strong SL feedback response in *max3-9* since branch lengths were less diminished than *max2-1* in comparison to wt. The altered hypocotyl epidermis cell number in the signalling mutant and different responses to GR24 also gave a considering indicator for alternative pathways. Low correlative inhibition could be found in max mutants based on levelled off gradients in secondary branch lengths in proximal direction.

A Comparison of secondary bud break to secondary branch growth demonstrated the importance in differentiating between bud break and branch outgrowth. This also indicated an enhancing impact of SLs on biomass allocation into broken buds instead of internode elongation. The difference between genotypes with relatively and absolutely more biomass allocation to the rosette in max mutants revealed a considerable pleiotropic effect of max mutations. A strong imbalance of sink and source strength in these due to unbridled meristem activity provided an indication on crossing a tipping point, at which shoot biomass controversially is decreased in response to higher sink number and therefore strength. Total branch length could probably have been bigger than in the wt if the redundancy of excessive rosette leaf growth and proximal branching was absent. This extensive investment in outgrowth of buds in vegetative metamers was grounded on the expense of internode elongation and generative phytomers, which were negligibly diminished in *max3-9*, while *max2-1* mutants possessed less than half of those of Col-0 at 76 d (data not given), indicating another great difference between these mutants.

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Chapter 6 - Appendix

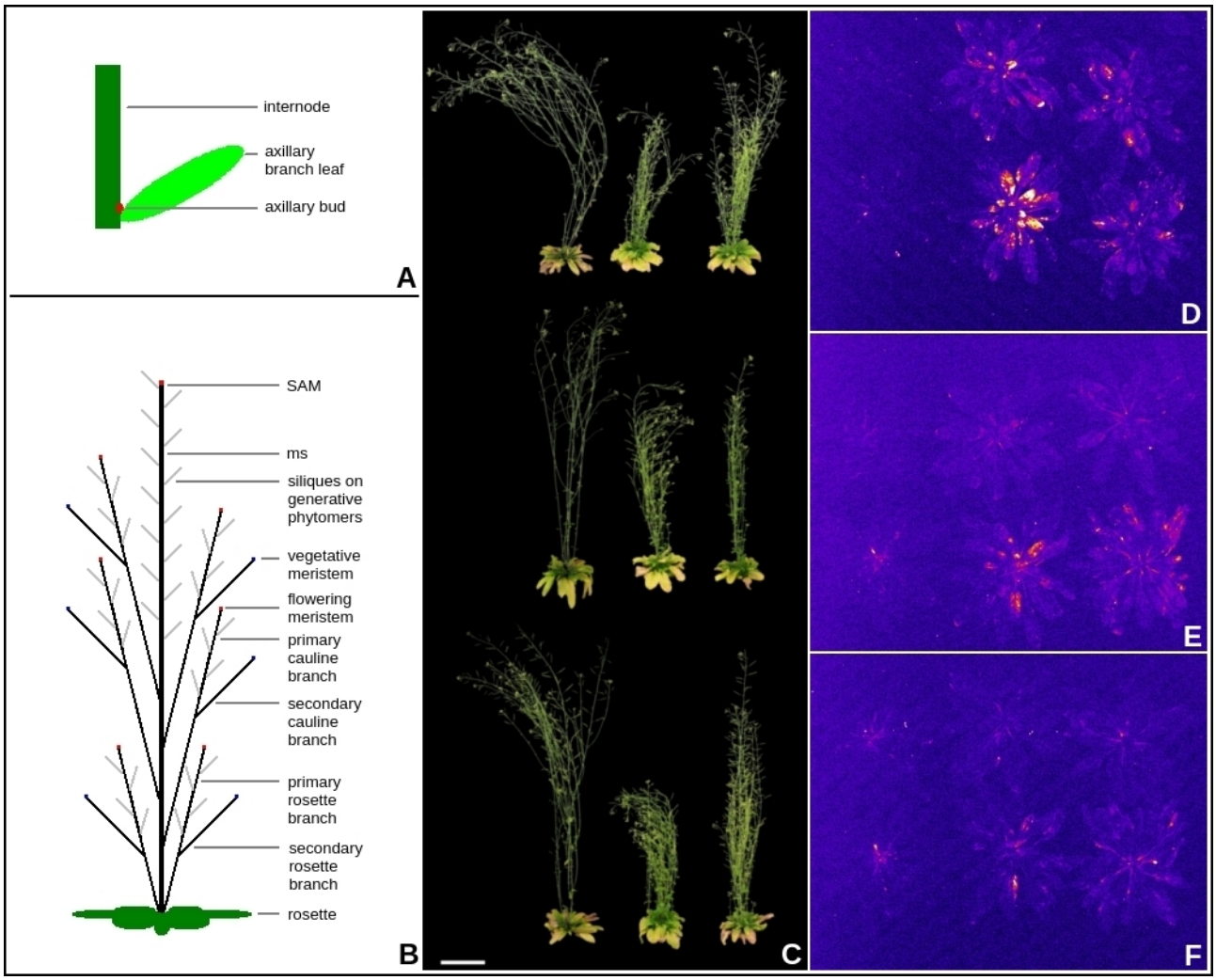

Fig. A.1. Plant model, plant morphology and starvation Luc reporter plants. (A) Arabidopsis vegetative phytomer, the basic component of shoot structure. (B) Plant model, declaration of plant architecture. (C): Plants after 75 d before destructive analysis. Control treatment, extended night, Tre application (top-down), Plants were stabilised in an erect posture for these photos. Col-0, *max2-1*, *max3-9* (from left to right). Scale bar indicates 10 cm. (D-F) Luminometer photos of 46-48 d old starvation Luc reporter plants. (D) Subsequently after extended night (+2 h), (E) 1 d later 2 h after night and (F) 2 d later, 2 h after night. Treatments: control, extended night, extended night with Tre application (in double rows, from left to right). Brightness of luciferase activity (fluorescent emission) was adjusted and illustrated by using the ImageJ lookup table “Fire”, grading activity from low to high (blue, red, yellow).

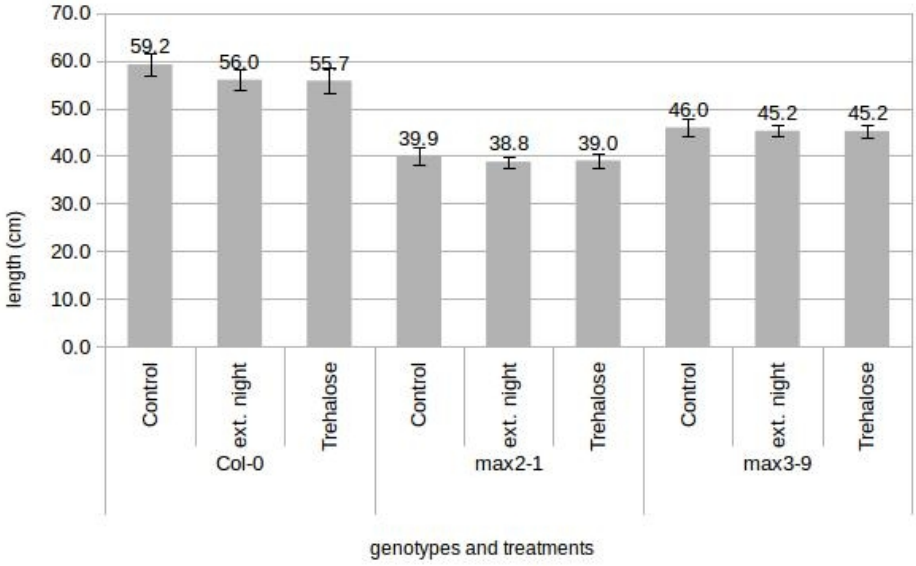

Fig. A.2. ms length after 76 d. Bars and labels show mean values with corresponding *SE* (n=15).

Tab. A.1. Significances of pairwise comparisons of ms length (n=15).

Treatment	Control	Control	Control	ext. night	ext. night	ext. night	Trehalose	Trehalose	Trehalose
Comparison	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*
P [ab]	***	**	*	***	***	**	***	**	**
Genotype	Col-0	Col-0	Col-0	*max2-1*	*max2-1*	*max2-1*	*max3-9*	*max3-9*	*max3-9*
Comparison	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night
P [ab]									

[a] *Signif. codes: P <0.001 '***', <0.01 '**', <0.05 '*'.*
[b] *Signif. normally distributed.*

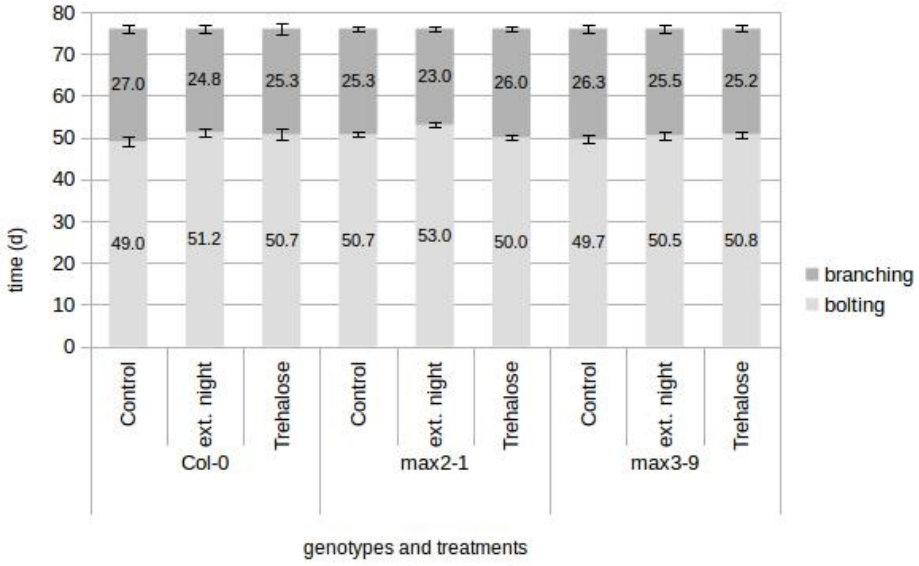

Fig. A.3. Period until bolting and period of branching until destructive analysis after 76 d. Bars and labels show mean values with corresponding *SE* on top (n=15).

Tab. A.2. Significances of pairwise comparisons of period until bolting and period of branching (n=15).

Treatment	Control	Control	Control	ext. night	ext. night	ext. night	Trehalose	Trehalose	Trehalose
Comparison	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*
P [ab] bolting	*			*		*			
P [ab] branching	*			*		*			
Genotype	Col-0	Col-0	Col-0	*max2-1*	*max2-1*	*max2-1*	*max3-9*	*max3-9*	*max3-9*
Comparison	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night	ext. night, Trehalose	Control, Trehalose	Control, ext. night
P [ab] bolting				***		**			
P [ab] branching				***		***			

[a] *Signif. codes: P <0.001 '***', <0.01 '**', <0.05 '*'.*
[b] *Signif. normally distributed.*

Fig. A.4. Rosette development of Col-0, *max2-1* and *max3-9* (top to bottom) under control conditions weeks 3-7 (left to right). Varying illumination of photos was adjusted to brightness. Scale bar indicates 1 cm.

Fig. A.5. Isolated *max2-1* (upper row) and *max3-9* (lower row) rosettes after 76 d, top view (left), bottom view (right). Red arrows show young rosette branch leaves, present in an obvious higher number than rosette branches. Scale bar indicates 1 cm.

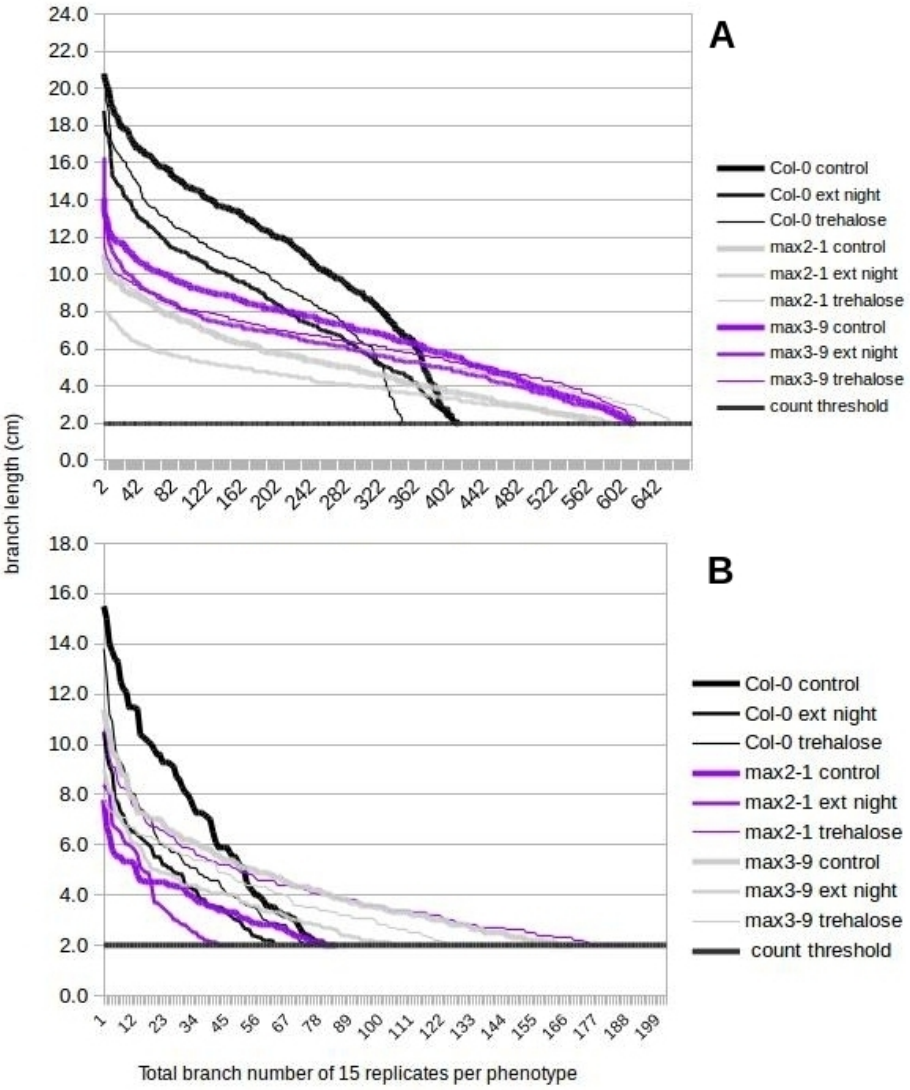

Fig. A.6. Total sum of secondary cauline (A) and secondary rosette (B) branch lengths of 15 replicates each. The interval below curves (sum of secondary branch lengths in controls fig. 3.4) of Col-0 is bigger than those of the mutants apart from other treatments, while branch lengths of mutants are more even distributed and these possess a higher number of branches with a length above the count threshold (intercept at 2.0 cm). Nevertheless, fig. 3.2, fig. 3.3 indicate the presence of a high number in secondary cauline branches in Col-0, with lengths below the mentioned threshold.

DNA extraction

Extraction buffer: 0.1 M TrisCl (pH 8.0), 0.5 M NaCl, 0.05 M EDTA, add before use: 0.01 M ß-mercapto-ethanol (Sigma 63689) (1.0 vol. %). CTAB-buffer: 0.2 M TrisCl (pH 7.5), 2 M NaCl, 0.05 M EDTA, 2% (w/v) CTAB.
10% SDS, isopropanol, 95% ethanol, T10E1 buffer, pH 8.0, chloroform/isoamylalcohol (24:1), RNAse (10 mg ml^{-1}, box modified enzymes).

1. Grind leaves (100-200 mg) in Eppendorf tube in liquid nitrogen
2. Add 1 ml extraction buffer and 50 µl 10% SDS. (Mix by rotation)
3. Incubate for 30 min at 65 °C
4. Centrifuge for 10 min at 13200 rpm
5. Transfer supernatant to a new Eppendorf tube and add equal volume of isopropanol
6. Incubate for 10-30 min on ice
7. Centrifuge for 10 min at 13200 rpm
8. Discard supernatant, dissolve DNA pellet in 400 µl TE
9. Add 1 µl RNAse
10. Incubate for 1 min at 37 °C
11. Add 400 µl CTAB buffer and incubate for 15 min at 65 °C, mix from time to time (every 5 min)
12. Add 800 µl chloroform/isoamylalcohol and mix
13. Centrifuge for 5 min at 13200 rpm
14. Transfer upper phase to fresh 2.2 ml Eppendorf, add 1.4 ml ethanol (95%; JB pure ethanol) and incubate at RT for 15 min
15. Centrifuge for 10 min at 13200 rpm, discard supernatant and wash pellet with 70% ethanol
16. Re-centrifuge and remove ethanol (leave tubes open or centrifuge for 4 min at 400 rpm in open tubes
17. Dissolve in 100 µl MQ
18. Measure concentration on nanodrop (1 µl)

Tab. A.3. Primers for PCR analysis, designed according to Booker *et al.* (2004).

Primer	Sequence
max2-1_wt_fw	CTATAGGGAGAGGATGTTGTAAG
max2-1_wt_rev	AATCTTTCCCATAAACTCAAATC
max2-1_mut_rev	AATCTTTCCCATAAACTCAAAT**T** [a]
max3-9_mut_fw	CACCGCTAAAATCTCCACCG
max3-9_mut_rev	TGCGGCTGTGTCCCATATAT
max3-9_rev_wt	TGAGTATCCGTGAATGCCCA
max2-1_seq_fw [b]	GTTGTCTCGCTCTCTACCCA
max2-1_seq_rev [b]	TGGCCAATAATCAAGCTCGC

[a] The point mutation in the *max2-1* mutant is indicated with a bold letter.
[b] Primers for additional PCR for point mutation in the isolated *max2-1* fragment (using Q5 proofreading polymerase).

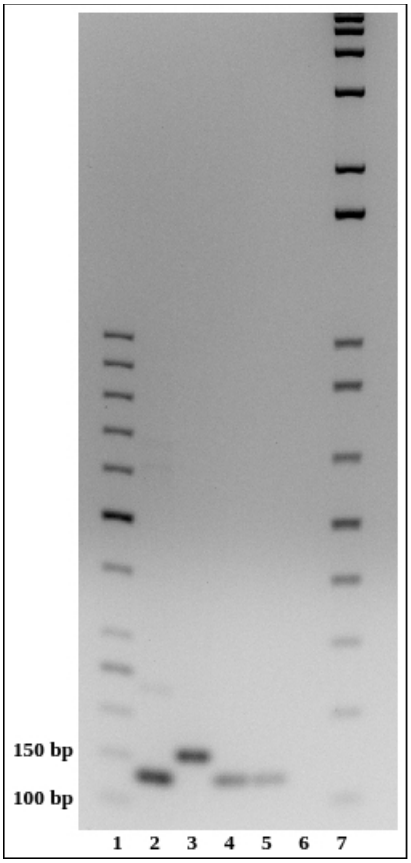

Fig. A.7. PCR results of Genotype analysis, disconfirmation of the putative *max2-1/max3-9* double mutant. Configuration of gel-electrophoresis: (1) 50 bp ladder, (2) Col-0 (PL 15001), (3) *max3-9* (PL 13013), (4) *max2-1/max3-9* (PL 15002), (5) *max2-1/max3-9* (PL 15003) (6) MQ neg. control, (7) Ready-Load 1 kb Plus DNA ladder marker. DNA analysis was conducted to verify the putative *max2-1/max3-9 mutant* was a pure bred of the pure *max2-1* and *max3-9* mutants and no wt Col-0 progenitor, as shown in figure 3XX. The mutation in *max3-9* originates from a deletion of 16 nucleotides in the second exon, which is replaced by a 42 nucleotides fragment of unknown origin (Booker *et al.* 2004). Therefore, the mutant fragment is 26 bp longer , so the second exon has a length of 155 (129 + 26) bp compared to the WT PCR fragment (129 bp). According to the PCR result (figure 3.XX), it was confirmed the MAX3-9 mutation is not present in either of both putative *max2-1/max3-9* lines, which were assumed to be homozygous double mutants. The attempt to verify a MAX2-1 background failed since no differentiation in primer matching occurred in consequence of the point mutation (max2-1 wt rev AATCTTTCCCATAAACTCAAATC, max2-1 mut rev AATCTTTCCCATAAACTCAAATT). Based on the high phenotypical conformity of the putative double mutant (PL 15002) with the *max2-1* mutants, this mutation was assumed to be existent is this line.

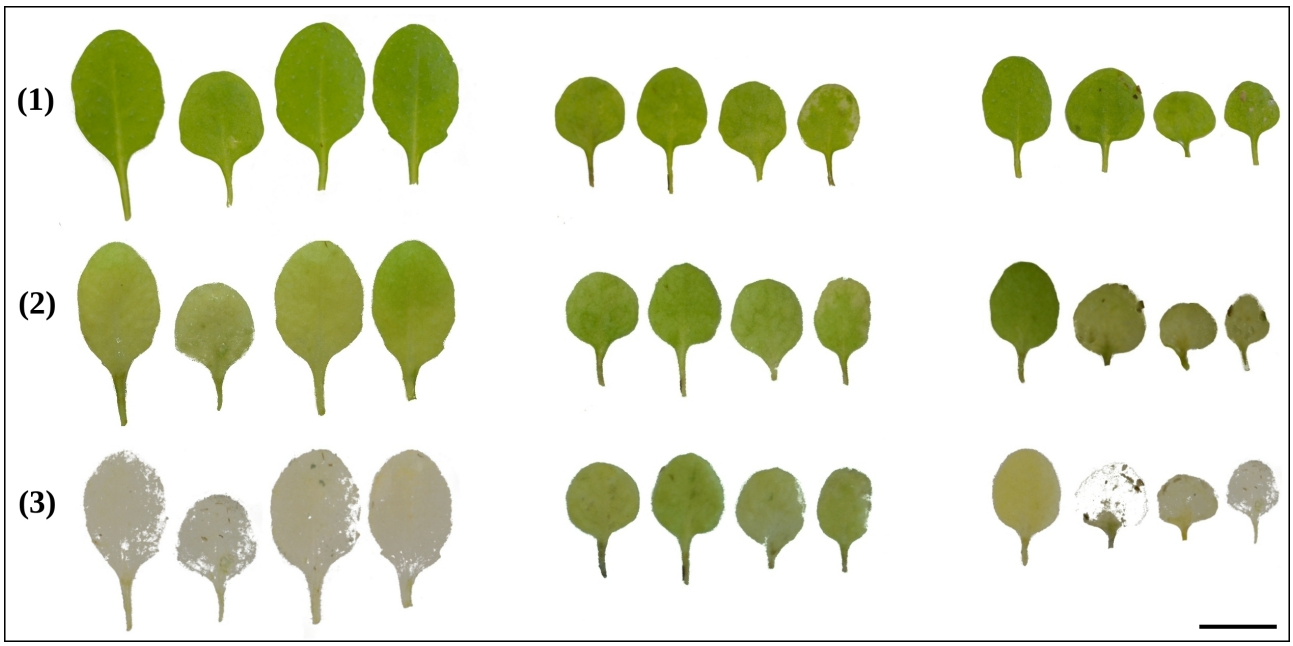

Fig. A.8. Senescence progression of isolated, 20 d old, soil grown leaves, transferred on sealed agar plates. Photos were taken (1) before, (2) 6 d and (3) 11 d after etiolation, Col-0, *max2-1*, *max3-9* (columns left to right). Scale bar indicates 1 cm.

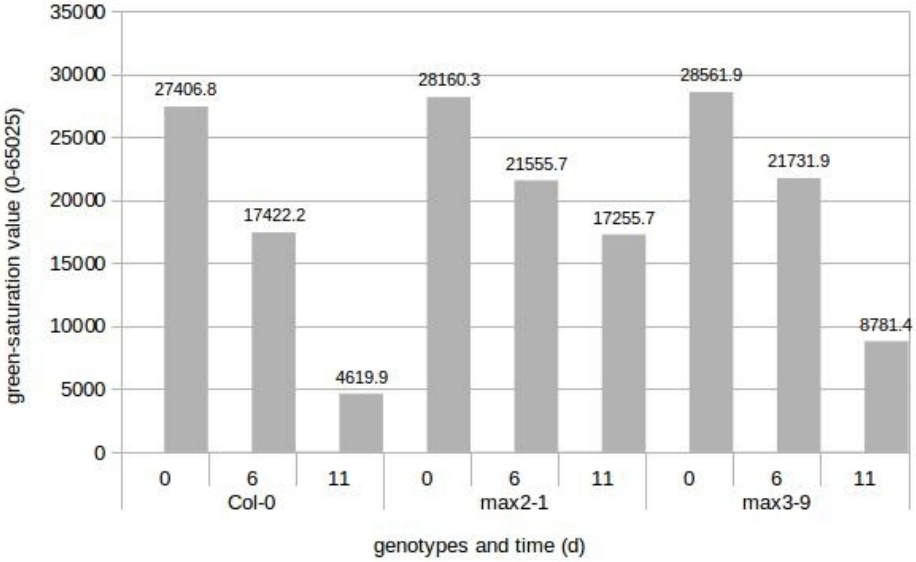

Fig. A.9. Product of RGB green values and HSV saturation value of isolated, 20 d old leaves over time. Both values separately in the next figures. Bars and labels show mean values (n=20).

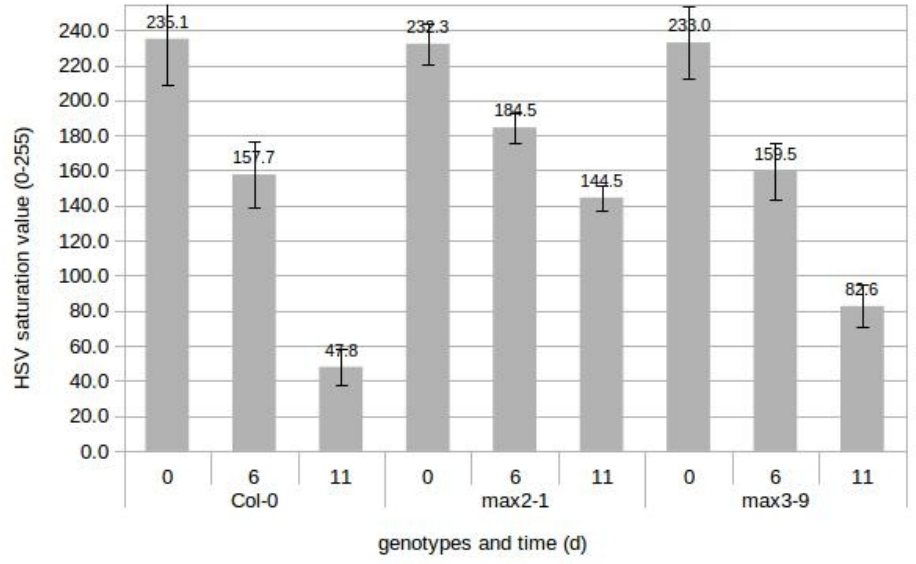

Fig. A.10. HSV saturation values of 20 d old isolated leaves, subsequently etiolated for 11 d over time. Bars and labels show mean values with corresponding *SE* (n=20).

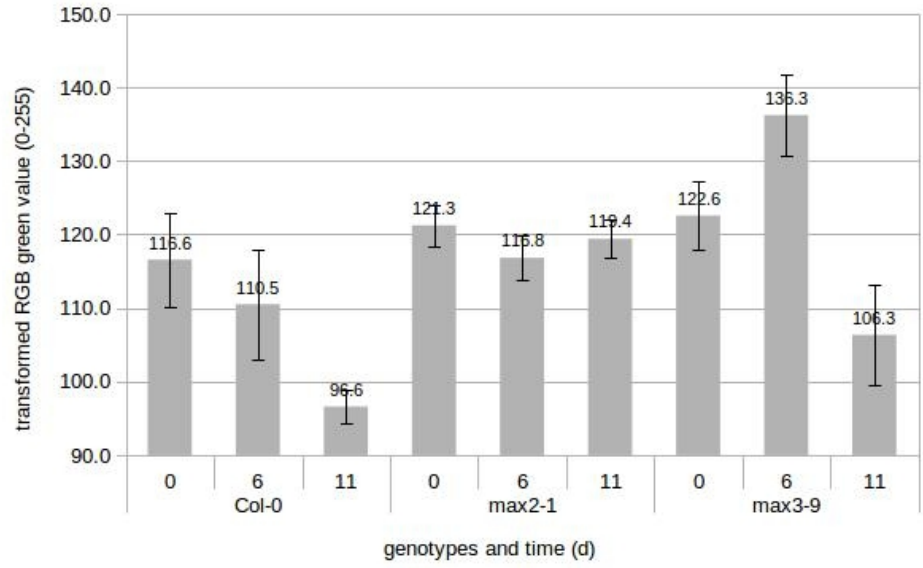

Fig. A.11. RGB green values of 20 d old isolated leaves, subsequently etiolated for 11 d over time. Green values were inverted (255 - value$_i$), since 0 corresponds to the maximum. Bars and labels show mean values with corresponding *SE* (n=20).

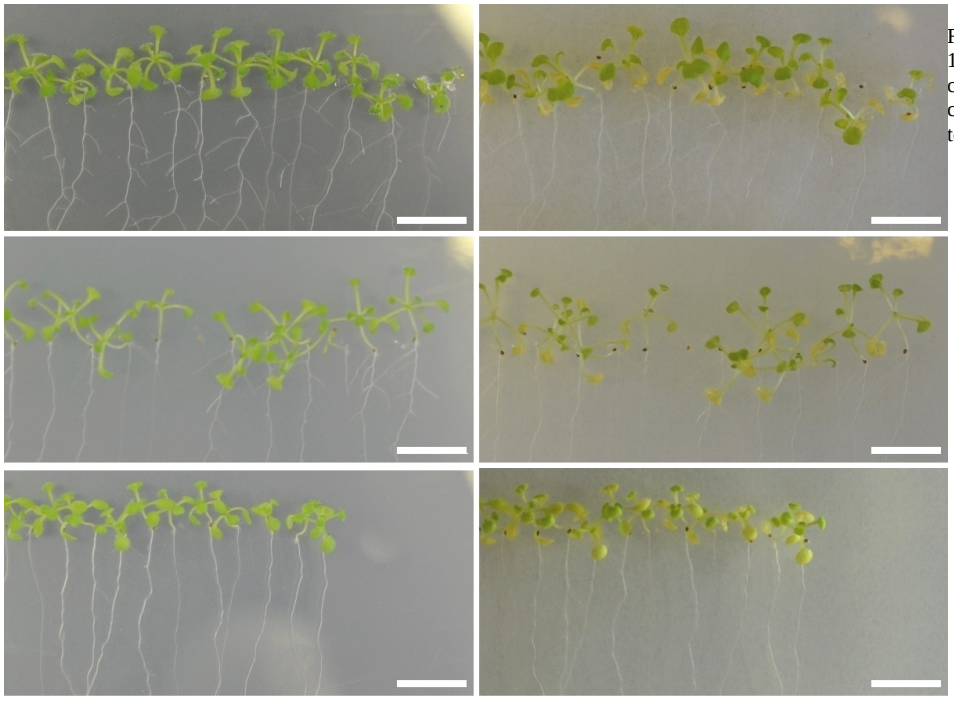

Fig. A.12. Senescence progression of 13 d old, illuminated seedlings (left column) and after 12 d etiolation (right column). Col-0, *max2-1*, *max3-9* (rows top-down). Scale bars indicate 1 cm.

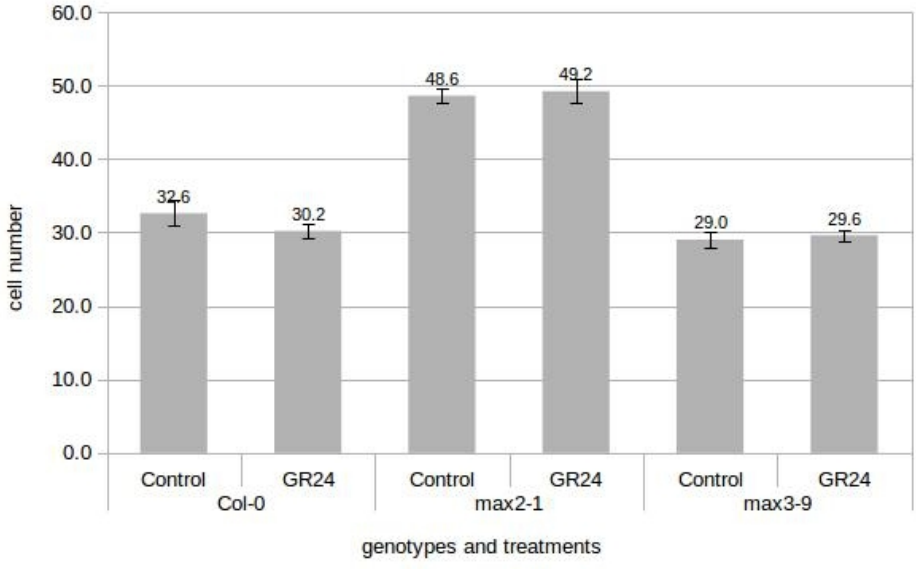

Fig. A.13. Hypocotyl epidermis cell number of 7 d old seedlings treated with 5 µmol GR24 and control (DMSO) treatment. Bars and labels show mean values with corresponding *SE* (n=5). No significant difference in cell number between treatments was observed (tab. A.4).

Tab. A.4. Significances of pairwise comparisons of hypocotyl epidermis cell number (n=5).

Treatment	Control	Control	Control	GR24	GR24	GR24	Col-0	*max2-1*	*max3-9*
Comparison	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Control, GR24	Control, GR24	Control, GR24
P [a]	**		**	*		*			

[a] *Signif. codes: P <0.001 '***', <0.01 '**', <0.05 '*'.*

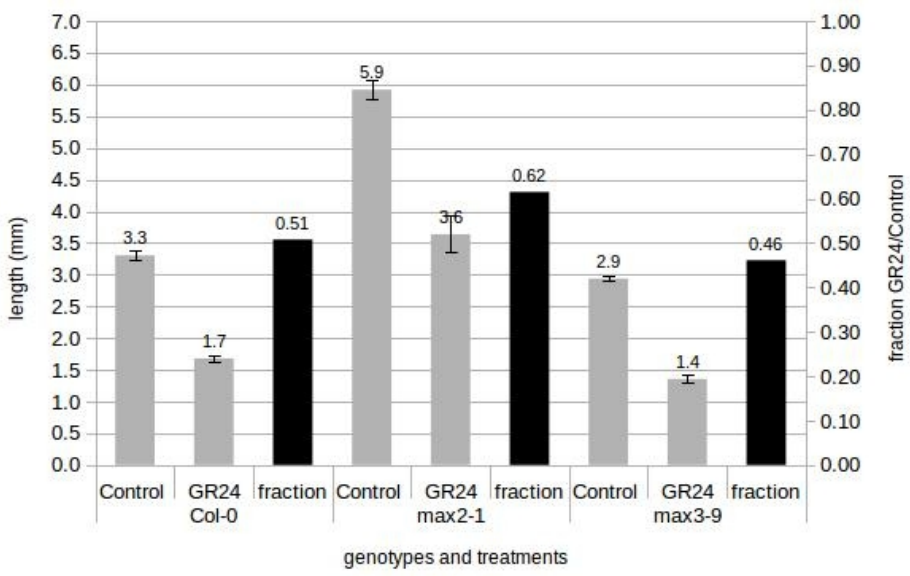

Fig. A.14. Hypocotyl lengths of 7 d old seedlings treated with 5 µmol GR24 and control treatment. The fraction in length of GR24 treated/Control (DMSO) plants is given as black bars, referring to the second y-axis. Bars and labels show mean values with *SE* (n=20).

Tab. A.5. Significances of pairwise comparisons of hypocotyl length (n=20).

Common factor	Control	Control	Control	GR24	GR24	GR24	Col-0	*max2-1*	*max3-9*
Comparison	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Control, GR24	Control, GR24	Control, GR24
P [abc]	***	***	***	***	***	***	***	***	***

[a] *Signif. codes: P <0.001 '***', <0.01 '**', <0.05 '*'.*
[b] *Signif. normally distributed.*
[c] *Signif. homogeneity of variances.*

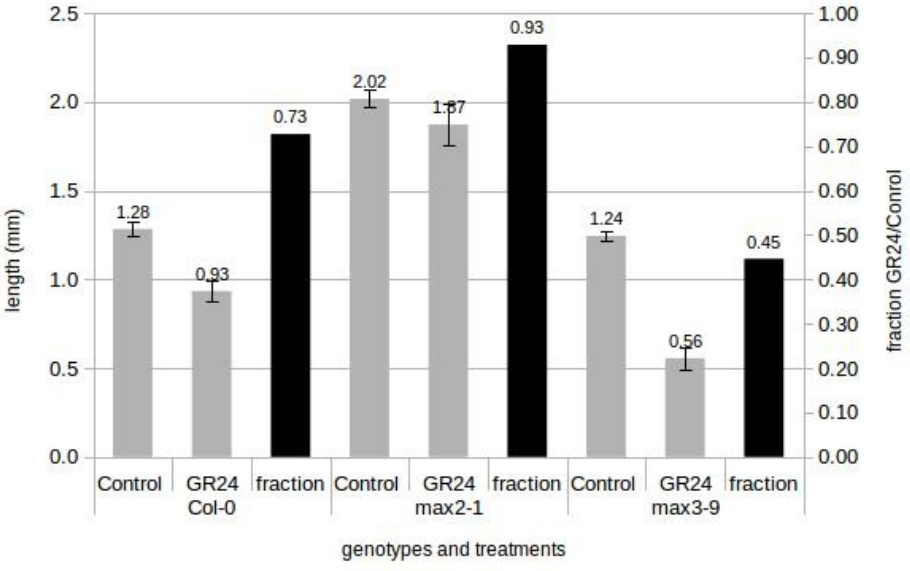

Fig. A.15. Petiole lengths of 7 d old seedlings treated with 5 µmol GR24 and control treatment. The fraction in length of GR24-treated to control (DMSO) plants is given as black bars, referring to the second y-axis. Bars and labels show mean values with corresponding *SE* (n=20).

Tab. A.6. Significances of pairwise comparisons of petiole length (n=20).

Common factor	Control	Control	Control	GR24	GR24	GR24	Col-0	*max2-1*	*max3-9*
Comparison	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Control, GR24	Control, GR24	Control, GR24
P [abc]	***		***	***	***	***	***	***	***

[a] *Signif. codes: P <0.001 '***', <0.01 '**', <0.05 '*'.*
[b] *Signif. normally distributed.*
[c] *Signif. homogeneity of variances.*

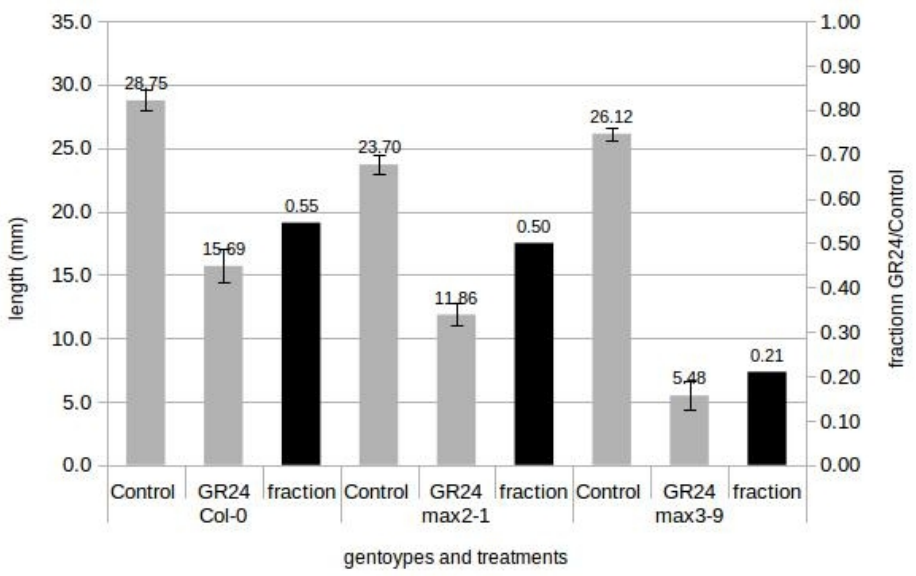

Fig. A.16. Primary root lengths of 7 d old seedlings treated with 5 µmol GR24 and control treatment. The fraction in length of GR24 treated/Control (DMSO) plants is given as black bars, referring to the second y-axis. Bars and labels show mean values with corresponding *SE* (n=20).

Tab. A.7. Significances of pairwise comparisons of primary root length (n=20).

Common factor	Control	Control	Control	GR24	GR24	GR24	Col-0	*max2-1*	*max3-9*
Comparison	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Col-0, *max2-1*	Col-0, *max3-9*	*max2-1*, *max3-9*	Control, GR24	Control, GR24	Control, GR24
P [ab]	**	*		**	***	***	***	***	***

[a] *Signif. codes: P <0.001 '***', <0.01 '**', <0.05 '*'.*
[b] *Signif. normally distributed.*

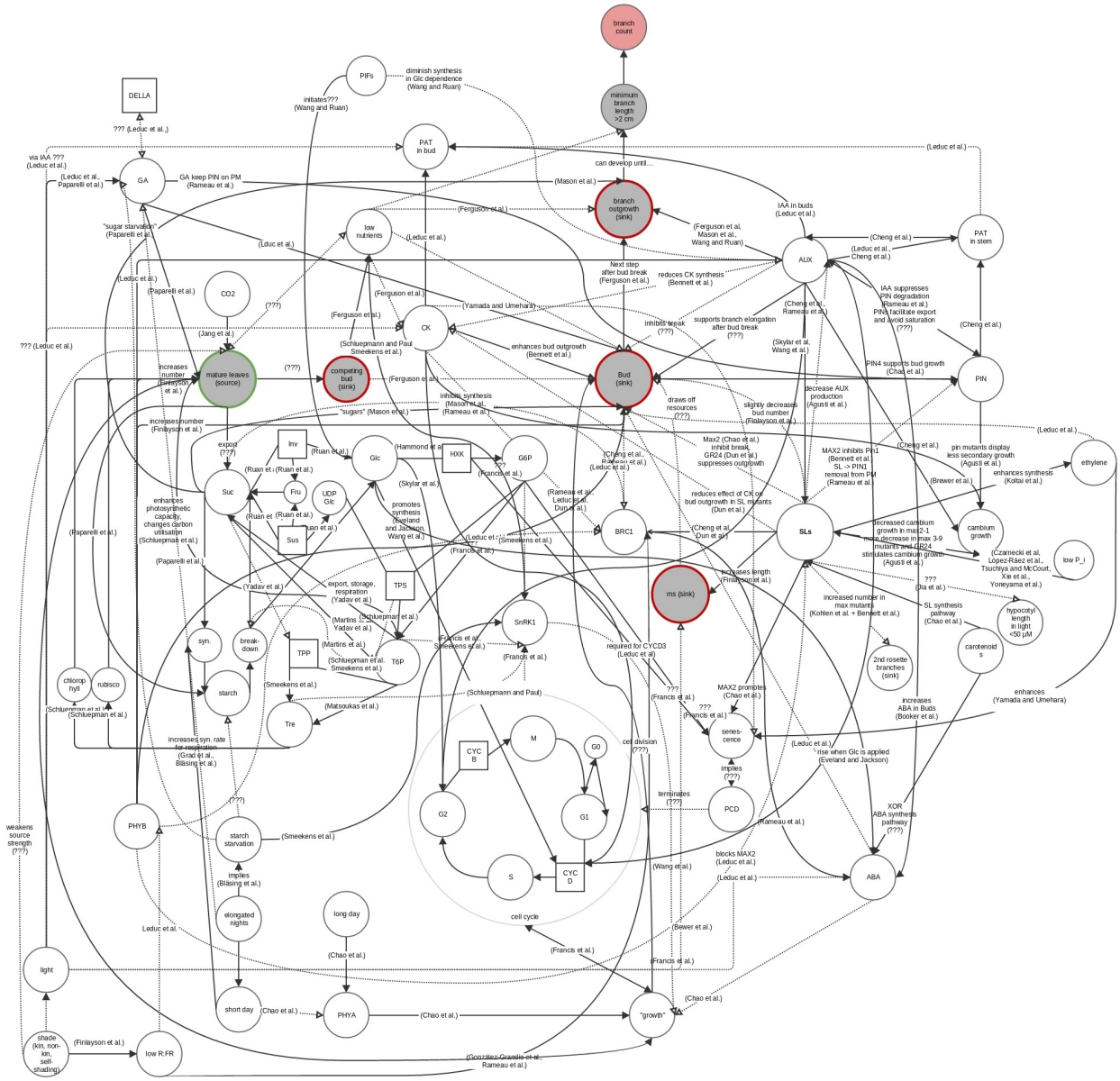

Fig. A.17. Overview diagram of factors, which have an important role in the process of branch development until count (pink circle). This model should picture the complexity of the branching process. Arrows with black tip show enhancing/positive effects, while arrows with dashed lines and white head indicate inhibition or negative effects.