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The bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of JA responses

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SYNOPSES

This work identifies two novel transcription factors, MYC3 and MYC4, as novel targets of JAZ repressors and regulators of responses to jasmonate. MYC3 and MYC4 act mostly redundantly with MYC2. However, MYC2 has a predominant role on root-growth inhibition by JA, whereas MYC3 and MYC4 have a predominant role on JA-mediated plant defence. This specificity can be explained by the differences on gene expression patterns.

SUMMARY

Jasmonates (JAs) trigger an important transcriptional reprogramming of plant cells to modulate both basal development and stress responses. In spite of the importance of transcriptional regulation, only one transcription factor (TF), the bHLH MYC2, has been described so far as a direct target of JAZ repressors. By means of yeast two hybrid screening and tandem affinity purification strategies, we identified two novel targets of JAZ repressors, the TFs MYC3 and MYC4, phylogenetically closely related to MYC2. We show that MYC3 and MYC4 interact *in vitro* and *in vivo* with JAZ repressors and also form homo- and heterodimers with MYC2 and among themselves. They both are nuclear proteins that bind DNA with sequence specificity similar to that of MYC2. Loss-of-function mutations in any of these two TFs impair full responsiveness to JA and enhance the JA-insensitivity of *myc2* mutants. Moreover, the triple mutant *myc2;myc3;myc4* is as impaired as *coi1-1* in the activation of several, but not all, JA-mediated responses such as the defence to bacterial pathogens and insect herbivory. Our results show that MYC3 and MYC4 are novel activators of JA-regulated programs that act additively to MYC2 to specifically regulate different subsets of the JA-dependent transcriptional response. This specificity of TF activity constitutes a clue to understand how the diversity of JA-regulated responses can be determined.

INTRODUCTION

The plant hormones jasmonates (JAs) are fatty acid derived oxylipins required for the regulation of multiple physiological aspects of plant growth, development and defence (Wasternack, 2007; Kazan and Manners, 2008; Browse, 2009; Chung et al., 2009; Pauwels et al., 2009). Thus, JAs are widely recognized as regulators of plant responses to environmental stresses such as pathogens and pests attack, wounding, ozone exposure and water deficit (Devoto et al., 2005; Browse and Howe, 2008). They are also important regulators of growth and developmental programs such as gamete development, cell cycle, root-growth, tendril coiling and senescence in many plant species (Pauwels et al., 2008; Zhang and Turner, 2008; Reinbothe et al., 2009; Yoshida et al., 2009). JAs are being recognized as important integrators of developmental and stress signals to modulate the allocation of resources to “grow” or to “defend” (Moreno et al., 2009; Robson et al., 2010).

Transcription is a major regulatory step in the activation of these responses and JAs trigger an important transcriptional reprogramming of the cells to switch the basal developmental programs into the necessary stress response program (Reymond et al., 2004; Devoto et al., 2005; Mandaokar et al., 2006; Pauwels et al., 2008). The signalling events that lead to transcriptional reprogramming are starting to be elucidated. Upon elicitation by exogenous or endogenous signals the hormone, (+)-7-*iso*-jasmonoyl-L-Isoleucine (also known as (3*R*,7*S*)-jasmonoyl-L-isoleucine or JA-Ile), is synthesized by JAR1 (Fonseca et al., 2009b; Suza et al., 2010; Wasternack and Kombrink, 2010). JA-Ile is perceived by a receptor complex formed by the protein COI1 and the JAZ repressors (Xie et al., 1998; Thines et al., 2007; Katsir, 2008; Fonseca et al., 2009b; Sheard et

al., 2010). COI1 is an F-box protein that participates in Skp1–Cul1–F-box protein (SCF)-type E3-ubiquitin ligase complex and is responsible for the recognition and recruitment of specific substrates. The hormone stimulates the specific binding of COI1 and JAZ proteins that leads to ubiquitination of JAZ by SCF^{COI1} and subsequent degradation by the 26S proteasome (Chini et al., 2007; Maor et al., 2007; Thines et al., 2007; Yan et al., 2007; Saracco et al., 2009). In the absence of the hormone, JAZ repressors bind to transcription factors (TFs) and prevent their activity by recruiting the general co-repressors TOPLESS and TOPLESS-Related proteins (TPL/TPR) through the interaction with the adaptor protein NINJA (Pauwels et al., 2010). Upon hormone accumulation and perception, degradation of JAZ repressors liberates TFs from NINJA and TOPLESS and initiates the transcriptional reprogramming of the cell and the activation of the JA-responses.

The key signalling events identified so far, however, still do not explain how the diversity of JA responses is specifically regulated. Identification of new TFs targeted by the JAZ repressors represent a necessary step to address this question, since MYC2/JIN1 is so far the only TF described as a direct JAZ target (Chini et al., 2007). MYC2 is a key bHLH TF regulating the expression of different subsets of JA-responsive genes (Boter et al., 2004; Lorenzo et al., 2004; Dombrecht et al., 2007). However, MYC2 can not be the only TF regulating JA responses since *myc2/jin1* mutants do not show a complete loss of JA-sensitivity. Besides MYC2, several TFs have been involved in specific aspects of JA-induced responses. These include TFs such as ERF1, WRKYs and MYBs among others (Fonseca et al., 2009a). However, their interaction with JAZs has not been reported so far. It has been speculated that the specific

interactions between JAZs and their respective (still unidentified) TF targets may be largely responsible for the diversity and specificity of JA responses upon different stimuli. However, this hypothesis remains to be formally demonstrated.

Here we show that at least two closely related bHLH TFs, MYC3 and MYC4, act additively with MYC2 in the activation of JA-responses. Similar to MYC2, both MYC3 and MYC4 are nuclear proteins that interact *in vitro* and *in vivo* with JAZ repressors. MYC3 and MYC4 share similar DNA-binding specificities with MYC2 and form homo- and hetero-dimeric complexes among them and with MYC2 *in vivo*. Moreover, both TFs are required for full responsiveness to the hormone in several JA-regulated physiological processes including gene expression, inhibition of root-growth and pathogen and insect resistance. Indeed, Arabidopsis plants lacking simultaneously *MYC2*, *MYC3* and *MYC4* genes are as impaired as *coi1-1* in the activation of JA-mediated gene expression and defence responses to microbial pathogens and insect herbivory, indicating that MYC2, MYC3 and MYC4 may form a cluster of TFs controlling specific JA-dependent responses.

RESULTS

Identification of new targets of JAZ repressors

Yeast-two-hybrid screens using JAZ2 and JAZ3 as baits identified a total of 60 positive clones. Direct sequencing revealed that about 20% corresponded to

MYC2, confirming previous reports (Chini et al., 2007; Melotto et al., 2008; Chini et al., 2009). Sequencing of the remaining clones allowed the identification of two novel candidate JAZ targets as the TFs MYC3 (At5g46760) and MYC4 (At4g17880).

MYC3 and MYC4, together with At5g46830, are the closest homologues of MYC2 in the Arabidopsis genome (Supplemental Figure S1). To test if these MYC proteins interact with other JAZ proteins and if there is specificity in this interaction, we checked all possible combinations between all 12 JAZ proteins (as baits) and all four MYC TFs (as preys) by yeast-two-hybrid assays (Figure 1). Consistent with previously reported data, MYC2 interacts with virtually all JAZ proteins with the exception of JAZ4 and JAZ7 (Figures 1, S2 and (Chini et al., 2009; Melotto et al., 2008). Similarly, MYC3 interacts in yeast with all JAZ proteins except JAZ4 and MYC4 interacts with all JAZ, but very weakly with JAZ4. Thus, in contrast to MYC2, MYC3 and MYC4 interact with JAZ7, and MYC4 can weakly interact with JAZ4. In addition to this qualitative difference, quantitative differences on the intensity of interactions were also appreciated (Figure 1).

To further support that MYC3 and MYC4 are direct targets of JAZ proteins we performed pull-down (PD) experiments using recombinant purified MBP-JAZ (maltose-binding-protein-JAZ) fusion proteins and extracts of transgenic plants expressing MYC3 or MYC4 derivatives (MYC3-HA or MYC4-GFP). As shown in Figure 2, results were consistent with those in yeast. MYC3 and MYC4 could be pulled-down by most MBP-JAZ proteins, although again, qualitative and quantitative differences could be appreciated among different JAZ proteins and between MYC2, MYC3 and MYC4 (Figure 2 and Chini et al., 2009).

In spite of being the closest phylogenetic homolog to MYC2, At5G46830 was not identified in our screens (neither in yeast-two-hybrid nor in TAP-tagging in cultured cells; see below). Consistently, direct testing of the interaction of At5G46830 with the 12 JAZ proteins both in yeast-two-hybrid and PD experiments did not render any positive result (Figure 1 and Supplemental Figure 2).

To further confirm their interaction with JAZ proteins *in planta*, we performed tandem affinity purification (TAP-tagging) of protein complexes in cultured PSB-D Arabidopsis cells using MYC2, MYC3 and MYC4 as baits. As expected for a direct target of JAZ repressors (Chini et al., 2007), MYC2-TAP allowed the co-purification of several JAZ proteins including JAZ2, JAZ11 and JAZ12 (Table 1 and Supplemental Table S1). Moreover, MYC2-TAP also co-purified NINJA, which has been described as an adaptor protein between JAZ repressors and the co-repressors TOPLESS and TOPLESS-related (TPR) proteins (Pauwels et al., 2010). Therefore, these results indicate that MYC2 can form complexes *in vivo* with JAZ repressors and NINJA, as previously proposed (Chini et al., 2007; Pauwels et al., 2010). More interestingly, MYC3-TAP and MYC4-TAP baits also identified NINJA and several JAZ proteins (JAZ2 and JAZ12 in the case of MYC3 and JAZ2, JAZ11 and JAZ12 in the case of MYC4; Table 1). These results strongly support that these two new MYC TFs, similarly to MYC2, can form complexes with JAZ repressors and NINJA *in vivo*, and therefore, participate in JA-signalling modules. In addition to JAZs and NINJA, these TAP-tagging screens also identified MYC3 as an interactor of MYC2 and MYC4, and MYC4 as an interactor of MYC3, suggesting that they can form heterodimers *in vivo* (Table 1 and Supplemental Table ST1).

As an alternative method to identify new JAZ-interacting TFs we performed TAP-tagging screens using JAZ3 and JAZ5 as baits. Consistent with previous results (Chini et al., 2009; Pauwels et al., 2010), these baits identified NINJA, several JAZs, TPL and TPR proteins, confirming that these two JAZ proteins (JAZ3 and JAZ5) can participate in JA-signalling modules *in vivo* (Table 1, Supplemental Table ST1). Interestingly, both baits identified MYC3, further supporting that interactions with this TF occur in living cells (Table 1 and Supplemental Table ST1).

It is noteworthy that peptides corresponding to MYC2 were also identified in several TAP-tagging experiments (using JAZ3, JAZ5, MYC3 or MYC4 as baits). However, the statistical significance was below cut-off values, indicating that endogenous expression of *MYC2* or accumulation of the MYC2 protein may be very low in PSB-D Arabidopsis cultured cells.

The JAZ interaction domain of MYC proteins

To further characterize the domain in MYC proteins responsible for the interaction with JAZ repressors we performed yeast two-hybrid analysis using full-length MYC2 or several MYC2-derivatives (fused to the GAL4 activation domain) and JAZ1 or JAZ3 (fused to the GAL4 DNA-binding domain). As shown in Figure 3A, the full-length MYC2 protein and all derivatives that included the N-terminus were able to interact with both JAZ1 and JAZ3. However, derivatives lacking this N-terminus did not interact with any of these JAZs. The smallest positive MYC2 fragment tested had three regions also conserved in MYC3 and MYC4, the MYC activation domain (in black) and two additional conserved regions represented with grey vertical or horizontal stripes in Figure

3 and Supplemental Figure 3. To further delineate the MYC2 interaction domain, we separated these two conserved domains and tested the corresponding protein derivatives (MYC2-D^{93-160} and MYC2-D^{55-99} fused to the GAL4 DNA-binding domain; Figure 3B) in yeast two-hybrid assays against all MYC2-interacting JAZ proteins (fused to the GAL4 activation domain). The results showed that the MYC2-D^{93-160} region was sufficient for the interaction with most JAZ, whereas the other conserved domains were dispensable. To verify that this new domain also mediates the interaction of additional MYC proteins with the JAZs, we tested the corresponding MYC3-derivative (MYC3-D^{82-141}) and confirmed that this region was also sufficient for the interaction of MYC3 with most JAZ proteins (Figure 3B).

The identified JAZ-interaction domain is conserved among MYC proteins from several plant species and the degree of conservation is very high among MYC2, MYC3 and MYC4 (Supplemental Figure 3). Using this conserved domain in a BLAST search we identified additional MYC proteins bearing it, and therefore, representing new candidate JAZ targets (Supplemental Figure 3).

Homo- and heterodimerization between MYC TFs

TAP-tagging results suggested that MYC2, MYC3 and MYC4 TFs could dimerize *in vivo*. To further test this hypothesis we transiently expressed these proteins in *Nicotiana benthamiana* leaves and checked all possible combinations by co-immunoprecipitation (CoIP) experiments. Consistent with TAP-tagging results, MYC2, MYC3 and MYC4 could form homo- and hetero-dimers, whereas none of them interact with the closely related MYC TF AtAIB (At2g46510) (Figure 4). MYC4/MYC4 and MYC4/MYC2 signals are weak

suggesting that strength of the interactions may be different for different MYC/MYC combinations

DNA-binding specificity of MYC3 and MYC4

The interaction of MYC3 and MYC4 with JAZ repressors *in vitro* and *in vivo* and their hetero-dimerization with MYC2 suggests that these three TFs may share redundant functions. To test this idea we first confirmed their predicted nuclear localization *in vivo* using GFP-fusions of both MYC3 and MYC4 proteins. As MYC2 (Lorenzo et al., 2004), both MYC3 and MYC4 are nuclear proteins (Supplemental Figure S4).

Next, we characterized the DNA-binding specificities of MYC3 and MYC4 and compared them with that of MYC2 (recently characterized by Godoy et al., in press). We determined the consensus DNA-binding site of MYC3 and MYC4 using a Protein-Binding-Microarray developed in our laboratory (PBM11; Godoy et al., in press; see supplemental information). The PBM11 contains all possible combinations of double-stranded 11mers (~4.2 million sequences) combined in ~240,000 oligonucleotides. The PBM11 is hybridized against the TF fused to MBP and the result of specific binding of the TF to its DNA-binding sites revealed by an antibody against MBP. This PMB11 has been successfully used for the determination of the consensus DNA-binding site of MYC2 and other TFs (Godoy et al., in press). As shown in Figure 5, the consensus binding sites obtained for MYC3 and MYC4 are strikingly similar to that of MYC2 (the G-box), including their preferences for 5' and 3'-end nucleotides (Figure 5A and 5B). In addition, we also analyzed E-scores (reflecting binding affinities) of all three MYC proteins for all the possible G-related variants generated by replacing

each nucleotide in the canonical G-box by the three remaining bases. In this analysis, we observed the highest E-scores for the elements G, T/G, G-like, G/A and G/C, indicating that the MYC proteins tested showed similar binding affinities. However, we observed that whereas MYC2 and MYC3 were undistinguishable, MYC4 showed lower affinity for the G-box variants (Figure 5C). These results indicate that MYC2 and MYC3 have almost identical DNA-binding specificities and, therefore, likely recognize similar targets *in vivo*. By contrast, MYC4, although showing a very similar binding affinity to the other two MYC proteins, may recognize a slightly different subset of target genes *in vivo*, at least in its homodimeric conformation.

Expression patterns of *MYC3* and *MYC4*

To get a further insight into *MYC3* and *MYC4* function we analyzed their expression patterns using fusions of their promoters to the GUS reporter in stable transgenic plants. Both *MYC3* and *MYC4* showed a strong expression in aerial parts of young seedlings (Figure 6, panels A and E). In the case of *MYC3* the expression was observed in all tissues of hypocotyls, cotyledons and leaves, whereas MYC4 was preferentially expressed in the vasculature. Similarly, both were expressed in developed roots (panels B and F), but MYC4 expression was restricted to vascular tissues. In young roots (panels C and G), expression of both of them was very weak. In adult plants (panels D and H), GUS staining was observed in most organs of the plant including stems, siliques, flowers and young leaves. No evident induction or changes in gene-expression patterns could be detected after JA treatment. Q-PCR analysis of *MYC3* and *MYC4* gene expression confirmed that MYC3 and MYC4 are only

very weakly induced by JA treatment (supplemental Figure 5). These expression patterns are consistent with available microarray data (www.genevestigator.com; Supplemental Figure 7).

Phenotypic characterization of *myc3* and *myc4* mutants

Our results suggest a role of MYC3 and MYC4 in the regulation of JA responses. To test this hypothesis we obtained T-DNA insertional mutants (from Gabi-Kat; www.gabi-kat.de) for both genes, selected homozygous plants and analyzed the response to JA of each single mutant, doubles (of both and with *myc2*) and triple mutants. We analyzed typical JA-regulated responses such as JA-dependent gene expression, root-growth inhibition and defence responses to insects and bacterial pathogens. Mutations in *myc3* or *myc4* affected to different degrees all tested responses to the hormone, as described below for each phenotypic analysis.

JA-dependent gene expression

We analyzed induction of JA-marker gene expression in 8 day-old WT and mutant seedlings. As JA-marker we chose genes induced at different times after JA treatment, i.e.: immediate early (*JAZ10*), medium (*VSP2*) and late (*PDF1-2*) expressed genes. As shown in Figure 7, both *myc3* and *myc4* single mutants exhibit significantly reduced JA-induction of *JAZ10* and *PDF1-2*. Interestingly, analysis of double and triple mutants revealed that each MYC TF has a striking additive effect on JA-inducibility of all tested marker genes (*JAZ10*, *VSP2* and *PDF1-2*), rendering the triple mutants almost as impaired in JA-dependent gene expression as *coi1-1*. Therefore, these results indicate that all three MYC2,

MYC3 and MYC4 positively contribute to the activation of gene expression in response to JA.

It is noteworthy that the single mutant *myc3* showed an enhancement of *VSP2* induction by JA, which reminds the negative effect of MYC2 on *PDF1-2* expression (Figure 7 and (Lorenzo et al., 2004)). This effect could suggest that MYC2 and MYC3 behave as repressors of *PDF1-2* and *VSP2*, respectively. However, the analysis of triple mutants discards this possibility since the triple *myc2;myc3;myc4* shows a lower expression of *VSP2*, for instance, than the double *myc2;myc4*. Gene expression analysis using Q-RT-PCR of the three MYC genes in each mutant background indicated that the enhancement of *PDF1-2* and *VSP2* in *myc2* and *myc3* mutants should not be caused by a compensatory enhancement of the expression of the other MYC genes (Supplemental Figure 6). However, whether this effect could be due to compensatory activation of the other two MYCs (e.g. by favouring particular dimeric combinations) or by enhanced expression of other unidentified MYCs, needs further study.

Root-growth inhibition by JA

Consistent with the low expression of MYC3 and MYC4 in young roots (Figure 6 and Supplemental Figure 7), but in contrast to the striking effect on JA-dependent gene expression in whole seedlings, *myc3* or *myc4* single mutants or the double *myc3;myc4* did not show any alteration in the inhibition of root-growth by JA compared to WT (Figure 8). In spite of this, the double *myc2;myc3* mutant and the triple *myc2;myc3;myc4* mutant showed a lower reduction of root-growth induced by JA than the single *myc2* mutant, indicating that *MYC3*

and *MYC4* also contribute to this JA-dependent phenotype. However, this contribution is weak since the triple mutant is significantly more sensitive to JA than *coi1-1*. These results indicate that MYC2 has a major role in the activation of JA responses in the root, whereas MYC3 and MYC4 have only a minor contribution in this tissue. This is consistent with the preferential expression of MYC2 in roots (Supplemental Figure 7). Therefore, other TFs are expected to participate in the regulation of JA responses in the root. In this context, we have already identified several MYC TFs bearing the conserved JAZ-interaction domain, which are good candidates to regulate these responses (Supplemental Figure 3).

Analysis of JA-dependent defence responses

The contrast between the strong effect of *myc3* and *myc4* mutants in gene expression analyses and the weak effect on root-growth inhibition was consistent with the low expression of MYC3 and MYC4 in young roots. Therefore, we studied JA-regulated defence responses that occur in aerial tissues, where MYC3 and MYC4 are strongly expressed. As an example of JA-dependent resistance we studied herbivory by the generalist herbivore *Spodoptera littoralis*, and as an example of JA-mediated susceptibility we analyzed the infection by *Pseudomonas syringae pv. Tomato* DC3000.

In spite of numerous replicate experiments, *myc2* mutant plants always showed a relatively modest increase in susceptibility to *S. littoralis* larvae (F. Schweizer, unpublished), which is consistent with its low expression in aerial tissues (Supplemental Figure 7). We thus hypothesized that MYC2 homologues might play a role in insect resistance. Indeed, single *myc3* and *myc4* mutants showed

a compromised resistance, each of them having a stronger effect than that of *myc2* mutants (Figure 9). Interestingly, the analysis of double mutant of *myc3* and *myc4* and triple mutants showed an additive phenotype rendering plants, in the case of the triple *myc2;myc3;myc4* mutant as susceptible as *coi1-1*, a loss-of-function allele of the hormone receptor which is completely insensitive to JA. These results are fully consistent with the gene expression analysis and indicate that each one of the three MYC TFs contribute to the activation of JA-dependent defences against *S. littoralis* with a prominent role of MYC3 and MYC4.

We also analyzed the response of the mutants to infection by the hemi-biotrophic pathogen *Pseudomonas syringae pv. tomato* DC3000. Contrary to insects that activate the JA pathway and trigger a strong defence response, this bacterial pathogen activates the JA pathway to promote susceptibility (Feys et al., 1994; Kloek et al., 2001; Laurie-Berry et al., 2006). Consistent with previous reports, the JA-insensitive mutants *coi1-1* and *myc2* showed increased resistance (both in terms of bacterial growth and leaf symptoms) that correlated with its level of JA-insensitivity (Figure 10A and 10B). Single *myc3* and *myc4* mutants showed an enhanced resistance (reduction of bacterial growth and leaf symptoms) to similar levels than those of *myc2*. As in the case of *S. littoralis*, analysis of double and triple mutants demonstrated an additive effect of all three genes rendering *myc2;myc3;myc4* mutant plants almost as resistant to Pto DC3000 as *coi1-1* (Figure 10A and 10B).

These results indicate that MYC3 and MYC4 have an additive effect to MYC2 in regulating JA-dependent responses mainly in aerial tissues.

Phenotypic analysis of MYC3/MYC4 gain-of-function

Overexpression of *MYC2* in transgenic plants promotes an enhanced responsiveness to JA (Figure 11; (Lorenzo et al., 2004)). To test whether, in addition to be required for full responsiveness to JA, MYC3 and MYC4 are also sufficient to activate JA-responses we analyzed the inhibition of root-growth by JA in transgenic lines ectopically expressing *MYC3* or *MYC4*. We chose to analyze root-growth inhibition because, as shown in Figure 6, the expression of these *TFs* in young roots is very weak, therefore making this tissue the best one to characterize the effect of their ectopic expression. As shown in Figure 11, in the absence of any treatment, 35S:*MYC3* lines showed a slight reduction in root-length compared to WT plants, similar to the phenotype of 35S:*MYC2* plants. In the presence of JA or coronatine the response of 35S:*MYC3* lines was also similar to that of 35S:*MYC2* transgenics showing shorter roots than WT controls. 35S:*MYC4*, however, did not show significant phenotypic differences with WT plants.

DISCUSSION

Activation of JA responses requires a profound transcriptional reprogramming of cellular genetic programs that involves a complex interplay between positive and negative regulators (i.e: TFs and JAZ repressors). Several TFs activating JA-responses have been described already but MYC2 was the only direct target of the JAZ repressors identified so far (Fonseca et al., 2009a). Nonetheless, MYC2 cannot be the sole JAZ-target for a number of reasons. Firstly, loss-of-function mutations in this TF do not affect all JA-dependent phenotypes. For instance, *myc2/jin1* mutants are fully fertile suggesting that other TFs should regulate this developmental process. Secondly, most JA-insensitive phenotypes associated to *myc2/jin1* are weaker than those of *coi1-1* (Feys et al., 1994; Lorenzo et al., 2004; Katsir, 2008; Fonseca et al., 2009b; Sheard et al., 2010). Thirdly, MYC2 negatively regulates the expression of some genes that are positively activated by JA, such as *PDF1-2* (Lorenzo et al., 2004; Dombrecht et al., 2007). Therefore, other direct targets of JAZ are expected to exist and the identification of such TFs has become a major task in the field.

Using several JAZ proteins as baits we identified two novel TFs, MYC3 and MYC4, phylogenetically closely related to MYC2, and show that both are direct targets of JAZ repressors and novel activators of JA-regulated programs that act additively to MYC2. Supporting these conclusions we show that MYC3 and MYC4 are nuclear proteins that bind DNA with similar specificity to that of MYC2. They interact *in vitro* and *in vivo* with several JAZ repressors and can also form homo- and heterodimers among them and with MYC2. Ectopic expression of MYC3 promotes a root phenotype similar (although weaker) to that of 35S*:MYC2* transgenic plants, suggesting that both MYC2 and MYC3 are

sufficient for activation of JA programs. Loss-of-function mutations in *MYC3* or *MYC4* impair full responsiveness to JA and enhance the JA-insensitivity of *myc2/jin1* mutants. Moreover, the triple mutant *myc2;myc3;myc4* is as impaired as *coi1-1* in the activation of several JA-mediated responses tested such as the susceptibility to hemibiotrophic pathogens (*P. syringae*), the resistance to insect herbivory (*S. littoralis*) as well as induction of JA-dependent gene expression (*JAZ10*, *VSP2* and *PDF1-2*). However, since other JA-regulated responses are not completely blocked (i.e.: inhibition of root-growth) or not altered at all (i.e. fertility) in the triple mutant, still additional TFs may be expected to act redundantly to MYC2, MYC3 and MYC4 to achieve full responsiveness to JA, at least in particular tissues or developmental processes.

A genetic screen for mutants altered in tryptophan metabolism identified *atr2D*, a dominant mutant in *MYC3* (Smolen et al., 2002). This mutant showed an enhanced expression of stress-related genes such *PDF1-2* and tryptophan-related genes that participate in the biosynthesis of indole-glucosinolates (Smolen et al., 2002). These *atr2D* phenotypes are consistent with our results and compatible with the activation of JA-mediated defence responses by a constitutively active form of MYC3. Interestingly, the mutation in *atr2D* affects a conserved amino acid (D94N), within the JAZ-interaction domain of MYC3. Whether this mutation prevents the interaction with JAZ proteins remains to be determined. However, this could be a plausible explanation for the constitutive MYC3 activity in the dominant *atr2D* mutant. Supporting the importance of the JAZ-interaction region, At5g46830, the closest homolog of MYC2, that does not interact with any JAZ proteins at all, bears a non-conservative amino acid

change (G to K) in this conserved region (equivalent to G91 in MYC3) (Supplemental Figure 3).

In addition to the identification of new TFs regulating JA-responses, one of the current major questions in the JA signalling field is how specificity and diversity of JA-responses is determined throughout the plant. In the case of auxins, this diversity seems to be a consequence of a combination of abundance and biochemical differences among TIR1/AFB auxin receptors, local concentration of bioactive auxins, the rate of degradation of AUX/IAA repressors and the tissue-specific expression patterns of the components of the auxin signalling modules (TIR/AFB receptors, AUX/IAA repressors and ARF TFs; (Dreher et al., 2006; Leyser, 2006; Muto et al., 2007; Mockaitis and Estelle, 2008; Parry et al., 2009; Vanneste and Friml, 2009)). In the case of JA, where only one receptor and one bioactive hormone has been described so far (Katsir, 2008; Fonseca et al., 2009b; Sheard et al., 2010), the rate of JAZ degradation and the differences in JAZ-MYC interactions, together with the tissue specificity of their expression patterns may determine how specificity is achieved in the regulation of the diversity of JA-regulated processes. Supporting this hypothesis, JAZ spliced isoforms are more stable than full-length JAZ proteins, which could potentially contribute to the strength and specificity of response (Yan et al., 2007; Chung and Howe, 2009; Chung et al, 2010). Moreover, in this work we provide evidence showing that part of this diversity and specificity can be explained by differences in MYC TFs function. MYC3 and MYC4 share functions with MYC2, but each of them seem to have a predominant role in particular processes. Whereas MYC2 has a major role on root-growth inhibition, MYC3 and MYC4 seem to be, for instance, more important than MYC2 in the regulation of

responses to herbivory. This suggests that MYC2, MYC3 and MYC4 are not fully redundant, but rather have evolved some specificity on their function. This specificity is likely due to the differences observed in their tissue expression patterns. However, we can not discard that the small variations on DNA-binding specificity and the differences observed in their affinities for JAZ repressors and among them (homo- and heteromeric interactions) may also contribute to the specificity of MYCs function.

Analyses of JA-marker gene expression in the single, double and triple mutants revealed that all three MYC proteins are required for full responsiveness to JA. In the case of immediate-early targets such as *JAZ10* all individual single mutants reduce inducibility by JA. In contrast, in the case of late-responsive genes such as *PDF1-2* and *VSP2*, single mutants (*myc2* and *myc3*, respectively) promote the opposite effect enhancing the response to the hormone. This would indicate that MYC2 and MYC3 act as repressors of *PDF1-2* and *VSP2* expression, respectively. However, the analyses of double and triple mutants clearly show that both of them (together with MYC4) are activators required for full responsiveness of these marker genes to the hormone. Therefore, our results show that over-induction of *PDF1-2* and *VSP2* in *myc2* and *myc3* mutants, respectively, is an indirect effect rather than a consequence of a direct repressive function of MYC2 and MYC3. This opens new perspectives on the understanding of MYC regulatory function and suggests that MYC TFs are subjected to a complex regulatory network of interactions where mutations in MYC2 or MYC3 promote unbalanced compensatory effects leading to this over-induction of *PDF1-2* and *VSP2*. Although it is tempting to speculate that this compensatory effect could be a

consequence of favouring particular dimeric combinations between the remaining MYCs in the mutant background that could interfere with their normal regulation, the molecular mechanism explaining this compensatory effect awaits further investigation.

Materials and Methods

Plant material and growth conditions

Arabidopsis thaliana Col-0 is the genetic background of wild-type and transgenic lines used throughout the work. Plants were growth in Johnson´s media at 21 °C under a 16-h light/8-h dark cycle as previously described (Lorenzo et. al, 2003).

The KO lines *myc3* (GK445B11) and myc4 (GK 491E10) were obtained from NASC. The 35S::MYC2-GFP was previously described (Chini et. al, 2009).

To generate transgenic plants expressing MYC3 or MYC4 in Col-0 background, full-length MYC3 and MYC4 coding sequences carrying or not stop codon were amplified with Expand High Fidelity polymerase (Roche, http://www.roche.com), using Gateway-compatible primers. PCR products were cloned into pDONR207 with a Gateway BP II kit (Invitrogen, http://www.invitrogen.com) and sequence verified. These plasmids, a Gateway LR II kit (Invitrogen) and the pGWB5 and pGWB14 (Mita et. al, 1995) destination vectors were used to generate 35S::MYC3, 35S::MYC3-GFP, 35S::MCY3-HA, 35S::MYC4, 35S::MYC4-GFP and 35S::MYC4-HA. These constructs were transferred to *Agrobacterium* t*umefaciens* strain C58C1 by freeze thawing and then transformed in Col-0 plants by floral dipping method (Clough and Bent, 1998) . Kanamycin and hygromycin resistant plants were selected, and their T2 progenies propagated for subsequent analysis.

Root measurements

For root-growth inhibition assays root length of 20 to 30 seedlings was measured 8 days after germination in presence or absence of 50 µM jasmonic acid (JA) (Sigma; http://www.sigmaaldrich.com) or 0.5 µM coronatine (COR) (Sigma). Three independent replicates (20 to 30 seedlings each) were measured for each sample. Values represent mean ± SD. Comparisons between double and triple mutants of *myc2*, *myc3* and *myc4* vs. *myc2* were done by one way ANOVA. Comparisons between OE lines and WT (Col-0) were done by Student's t test.

Yeast two-hybrid screen

JAZ2 and *JAZ3* sequences were PCR amplified with the Expand High Fidelity PCR system (Roche) from plasmid templates provided by TAIR as described. PCR products were digested with *Eco*RI and *Pst*I and cloned into *Eco*RI–*Pst*I digested pGBKT7 (with GAL4 DNA-binding domain; Clontech) and the constructs were sequence-verified. The pGBKT7-JAZ2 and pGBKT7-JAZ3 plasmids were used as baits and transformed into yeast strain Y187. The prey cDNA library, from *Arabidopsis* seedlings (12 days old) grown on P_i-starved medium, was prepared in the plasmid pGADT7 and in yeast mating strain AH109 following BD Matchmaker Library Construction (version PR32047; Clontech; http://www.clontech.com). Bait (Y187) and prey (AH109) were mated by growing 50 ml of bait and 500 µl of prey overnight on 2 × YPDA medium at

30 °C. Yeast diploids were selected by plating at 30 °C for 4 days on minimal medium SD lacking His, Leu, Trp and adenine and supplemented with 20 mM 3-aminotriazole. 67 and 38 clones containing putative interacting preys were selected for JAZ2 and JAZ3 baits respectively. These clones were then sequenced and confirmed.

Yeast two-hybrid assays

Full length MYC3, MYC4 and BHLH028 coding sequences carrying a stop codon were amplified with Expand High Fidelity polymerase (Roche, http://www.roche.com) using Gateway-compatible primers. PCR products were cloned into pDONR207 with a Gateway BP II kit (Invitrogen, http://www.invitrogen.com) and sequence verified. These constructs and the pDONR constructs previously described (Chini et al., 2009) were used in Gateway (Invitrogen, http://www.invitrogen.com) LR reactions, in combination with the destination low-copy yeast expression vectors pDEST22 (Gal4 AD) and pDEST32 (Gal4 BD), and were then checked by sequencing.

To assess protein interactions, the corresponding plasmids were co-transformed into *Saccharomyces cerevisiae* AH109 cells following standard heat-shock protocols (Chini et al., 2009). Successfully transformed colonies were identified on yeast synthetic drop-out lacking Leu and Trp. At 3 days after transformation, yeast colonies were grown in selective –Leu, –Trp liquid media for 6h and the cell density was adjusted to $3x10^7$ cellsmL^{-1} (OD600=1). A 3 µl sample of the cell suspensions was plated out on yeast synthetic drop-out lacking Ade, His, Leu and Trp and supplemented with 2mM 3-aminotriazole to test protein interaction. Plates were incubated at 28^0C for 2-4 days. The empty vectors pDEST22 or pDEST32 were also co-transformed as negative controls.

Protein extracts and pull-down assays

MBP-JAZ fusion proteins were generated as previously described (Chini et. al, 2009). Ten-day-old *Arabidospis thaliana* wild type seedlings, lines expressing 35S:MYC3-HA and 35S:MYC4-GFP were ground in liquid nitrogen and homogenized in extraction buffer containing 50 mM Tris-HCl pH 7.4, 80 mM NaCl, 10% glycerol, 0.1% Tween 20, 1 mM dithiothreitol, 1 mM phenylmethylsulphonyl fluoride (PMSF), 50 µM MG132 (Sigma), and complete protease inhibitor (Roche). After centrifugation (16,000*g* at 4 °C), the supernatant was collected. For *in vivo* pull-down experiments, 6 µg of resin-bound MBP fusion protein was added to 1 mg of total protein extract and incubated for 1 h at 4 °C with rotation. After washing, samples were denaturalized, loaded on 8% SDS–PAGE gels, transferred to nitrocellulose membranes and incubated with anti-HA-HR-peroxidase (Roche) or anti-GFP-HRP (Milteny Biotec, http://www.miltenybiotec.com) antibody.

A 3 µl aliquot of MBP-fused protein of each sample was run into SDS-PAGE gels and stained with Coomassie blue to confirm equal protein loading.

Tandem Affinity Purification

Cloning of transgenes encoding tag fusions under control of the constitutive cauliflower tobacco mosaic virus 35S promoter and transformation of Arabidopsis cell suspension cultures were carried out as previously described (Van Leene et al., 2007). Tandem affinity purification of protein complexes was done using the GS tag (Bürckstümmer et al., 2006) followed by protein precipitation and separation, according to Van Leene et al. (2008). For the protocols of proteolysis and peptide isolation, acquisition of mass spectra by a 4800 Proteomics Analyzer (Applied Biosystems), and MS-based protein homology identification based on the TAIR genomic database, we refer to Van Leene et al. (2010). Experimental background proteins were subtracted based on approximately 40 TAP experiments on wild type cultures and cultures expressing TAP-tagged mock proteins GUS, RFP and GFP (Van Leene et al., 2010).

Co-Immunoprecipitation

Nicotiana benthamiana leaves were infiltrated with *Agrobacterium* harbouring AtMYC3 and AtMYC4 proteins fused to GFP or HA tags. Empty pGWB vectors were used for the expression of GFP and HA proteins as negative controls. After 2 days from agroinfiltration 0.6 g of agroinfiltrated leaves were collected and homogenized in 2 ml of co-IP buffer containing 50mM Tris-HCl, pH 7.5, 100 mM NaCl, 2mM DTT, 0.1% Tween-20, 1mM PMSF, 50 µM MG132 and complete protease inhibitor cocktail (Roche), and were centrifuged twice at 16000*g* at 4^0C. The supernatant (1.5 mg of total protein) was incubated for 2 h (4^0C, with rotation) with the agarose conjugated anti-GFP matrix (MBL), and was washed three times with 1 mL of IP buffer. After denaturalization in 90 µl of Laemmli SDS-PAGE loading buffer, samples were loaded into 8% SDS-PAGE gels, transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, http://www.millipore.com) and incubated with anti-HA-HR-peroxidase (Roche) and anti-GFP-HRP (Milteny Biotec, http://www.miltenybiotec.com) antibodies. A 30 µl aliquot of total protein extract was also used for immunoblot with the same antibodies to confirm equal amount of recombinant proteins in each sample.

Protein purification and determination of MYC3 and MYC4 DNA-binding motifs

Translational fusions of MYC3 and MYC4 fused to Maltose Binding Protein (MBP) were obtained by cloning their corresponding cDNAs into the pTH1 vector using the Gateway technology. Donor templates were obtained through PCR amplification of pMYC3 and pMYC4 with oligonucleotides MYC3Fwgateway; MYC3Rvstopgateway (MYC3) and MYC4Fwgateway; MYC4Rvstopgateway (MYC4). Recombinant inserts were verified by sequencing and plasmids introduced into BL-21 strain. Expression and purification of recombinant proteins were as described in pMAL Purification System (New England Biolabs).

MYC3 and MYC4 DNA-binding specificities were determined using Protein Binding Microarrays (PBM11) as in Godoy et al. (in press). Briefly,

recombianant protein (1 ug) was incubated for 2.5 hours at room temperature onto a double-stranded DNA microarray containing all 11 bp sequences (~4.2 million) compacted in ~240,000 spots. After washes, mircroarrays were incubated with a primary antibody against MBP and a secondary antibody coupled with DyLight 549 fluorophore. Finally, slides were scanned at 5 μm in a GenePix 4000B scanner (Axon Instruments) and signal intensities quantified in the GenePix Pro 5.1 software. All the steps (synthesis *in situ* of double-stranded DNA, incubation and washes of recombinant proteins and antibodies, scanning, quantification and determination of DNA motifs) were performed as described (Godoy et al., submitted).

GUS staining

To generate transgenic plants expressing GUS protein under the regulation of *MYC3* and *MYC4* promoter regions, 2028 and 1549 bp, respectively, upstream of ATG (including the first 30 nucleotides of the coding sequence of each gene) were amplified with Expand High Fidelity polymerase (Roche, http://www.roche.com), using appropriate primers. PCR products were cloned into pENTR/D-TOPO (Invitrogen, www.invitrogen.com) and sequence verified. These clones, a Gateway LR II kit (Invitrogen) and pGWB3 destination vector were used to generate promoterMYC3:GUS and promoterMYC4:GUS. These constructs were transferred to *Agrobacterium* t*umefaciens* strain C58C1 by freeze thawing and then transformed in Col-0 plants by floral dipping method (Clough and Bent, 1998). 6 days-old seedlings or adult plant tissues from several T2 transgenic lines were stained for GUS activity. Samples were placed in staining solution 50 mM phosphate buffer (pH 7), 0.1 %(v/v) Triton (Sigma), 2mM X-Gluc (Glycosynth), 1mM K-ferrocyanide (Sigma), 1mM K ferricyanide (Sigma) and incubated at 37°C overnight. After staining, the tissue was soaked in several changes of 75% ethanol and keep in 5% glycerol until been photographed with a Leica DMR UV/VIS microscope (seedlings) or with a digital NIKON D1-x camera (adult plants). In the case of the roots, a root de-staining protocol was applied (Malamy and Benfey, 1997), 15 minutes incubation at 57^0C in 0.24N HCl, 20% methanol, 15 minutes incubation at room temperature with shacking in 7%NaOH, 60% ethanol. After these two incubations tissue was rehydrate washing in decreasing ethanol series and vacuum treated in 5%ethanol-25%glycerol.

Quantitative RT-PCR

Q-RT-PCR experiments were performed with RNA extracted from 1, 6 or 24 hours JA or mock-treated (DMF) seedlings. For each experiment three biological replicates, consisting of tissue pooled from 15-20 plants from different plates, were taken. RNA extraction and cleanup was done using Trizol reagent (Invitrogen) follow by RNeasy Mini Kit (Qiagen) and DNAse digestion to remove genomic DNA contamination. cDNA was synthesized from 0.5-1 μg of total RNA with High capacity cDNA reverse transcription kit (Applied Biosystems, http://www.appliedbiosystems.com). 5 μl from 1/10 diluted cDNA was used to amplify JAZ10 (1h treatement), VSP2 (6 hours treatment) PDF1-2 (24 hours treatment), and the housekeeping gene actin8 using Power SYBR Green (Applied Biosystems, http://www.appliedbiosystems.com). Quantitative PCR

was performed in 96-well optical plates in an 7300 Real Time PCR System (Applied Biosystems, http://www.appliedbiosystems.com). Thermocycler conditions comprised an initial holding at 50ºC for 120 sec then 95ºC for 10 min. This step was followed by a two-step SYBR PCR program consisting of 95ºC for 15 sec and 60ºC for 60 sec, for 40 cycles.

Insect bioassays

Arabidopsis thaliana (Col-0) and the mutants were vernalized in water for 4 days at 4˚C. *myc2;myc3;myc4* mutants were vernalized in water containing 0.1 mM gibberellic acid to stimulate germination. Seeds were then transferred to pots containing potting compost. *coi1-1* were germinated on Murashige and Skoog medium (Sigma, Buchs, Switzerland) containing 3% sucrose and 30 μM jasmonate and incubated under light (150 μmol $m^{-2}sec^{-1}$) for seven days in a growth chamber. Homozygous *coi1-1* mutants showing normal greening of leaves and no inhibition of root growth (Feys et al., 1994) were transferred to pots. Plants were grown for three weeks in a growth chamber as previously described (Reymond et al., 2000) .

Three week-old plants were placed in transparent plastic boxes in a growth chamber (20°C, 65% relative humidity, 100 μmol m^{-2} sec^{-1}, 10/14 h photoperiod). Forty newly hatched *Spodoptera littoralis* larvae were placed on seventy plants for seven days of feeding. Larvae were then collected and weighed. Data were analyzed on log-transformed values by Student's t-test. The experiment was repeated three times independently.

Bacterial assays on *Arabidopsis*

Pseudomonas syringae pv *tomato* (*Pto*) DC3000 growth assays in *Arabidopsis* were performed by spray inoculation. Briefly, overnight bacterial cultures were pelleted and resuspended in sterile 10 mM $MgCl_2$. Three to four week-old plants were sprayed with a bacterial suspension containing 10^8 (cfu)/ml bacteria (OD_{600}=0.2) with 0.04% Silwet L-77. Leaf discs were harvested after two days and ground in 10 mM $MgCl_2$. Population counts were performed at two days after infiltration. In both cases, serial dilutions of leaf extracts were plated on L agar with appropriate antibiotics. Each data point represents the average of 4 replicates, each containing two leaf discs from different plants. Error bars indicate standard errors of the mean (SEM). These experiments were repeated three times with similar results, and representative results are shown. Pictures of disease symptoms 3 days after inoculation on analysed genotypes (Col-0 (WT), *myc2 (2)*, *myc3 (3)*, *myc4 (4)*, *myc2;myc3 (2;3)*, *myc2;myc4 (2;4)*, *myc2;myc3;myc4*-1 (*2;3;4-1*),*myc2;myc3;myc4*-2 (*2;3;4-2*) and *coi1-1*) were taken with a digital NIKON D1-x.

Molecular phylogenetic analyses

Alignment of N- terminal JAZ interacting region of MYC2, MYC3, MYC4 and At5g46830 (BHLH028) from Arabidopsis and MYC2-closest relatives from Rice (AC060755.9), *Populus* (XM_002329476.1) and *Physcomitrella patens* (XP_001765161.1) and sequences of JAZ-interaction domain of MYC2, MYC3,

MYC4, At5g4683 (BHLH028), At5g41315 (GL3), At1g63650 (EGL3), At4g09820 (TT8), At1g01260 (BHLH013), At2g46510 (AtAIB) and At4g16430 (BHLH003), was carried out using a multiple alignment method with ClustalW algorithm (T-Coffee web server, http://tcoffee.vital-it.ch, default parameters, blosum matrix). Similarly, full-lenght protein sequence of 22 AtMYC2 bHLH-related proteins from Arabidopsis, Populus, Rice and *Physcomitrella* was used to generate a multiple alignment with ClustalW algorithm (T-Coffee web server, http://tcoffee.vital-it.ch, default parameters, blosum matrix). Phylogenetic tree of these 22 proteins was generated by MEGA4.0.2 using Maxima Parsimonia method (10000 bootstrap trials).

Accession Numbers

Sequence data from this article can be found in the *Arabidopsis* Information Resource (TAIR) or GenBank/EMBL databases under the following accession numbers: MYC 3 (locus AT5G46760; GenBank NM_124046.1), MYC 4 (locus AT4G17880; GenBank NM_117897.3), AtAIB (locus AT2G46510; GenBank NM_130216.2) and At5g46830 (locus AT5G46830; GenBank NM_124054.1).

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Supplemental Data

Table S1. MALDI-TOF/TOF-MS identification of MYC2, MYC3, MYC4, JAZ3 and JAZ5 interactors.

Table S2. List of primers used.

Figure S1. Phylogenetic tree of MYC2-related proteins from Arabidopsis and other plants.

Figure S2. Control growth of yeast cells transformed with MYC3 or MYC4 and with JAZ repressors in yeast two-hybrid assays.

Figure S3. Alignment of the JAZ-interaction domain of MYC2-related proteins.

Figure S4. MYC3 and MYC4 are nuclear-localized bHLH.

Figure S5. **JA dependent induction of *MYC3* and *MYC4* gene expression.**

Figure S6. Expression of MYC genes in *myc* mutant backgrounds.

Figure legends

Figure 1. MYC3 and MYC4 interact with JAZ repressors in yeast two-hybrid assays. Yeast cells co-transformed with pDEST22-MYC2, pDEST22-MYC3, pDEST22-MYC4 or pDEST22-bHLH028 (preys) and pDEST32-JAZ1-12 (baits) were selected and subsequently grown on yeast synthetic drop-out lacking Leu and Trp (-2), as a transformation control (shown in Supplemental Figure S2), or on selective media lacking Ade, His, Leu and Trp (-4), to test protein interactions (this figure). The lower panel show 1/10 dilution yeast growth in -4 selective media. pDEST22-MYC2, pDEST22-MYC3, pDEST22-MYC4 and pDEST22-bHLH028 co-transformation with pDEST32 vector were included as control.

Figure 2. MYC3 and MYC4 interact with JAZ repressors in Pull-Down experiments.

Immunoblots with anti-HA antibody of recovered MYC3-HA **(A)** or with anti-GFP antibody of recovered MYC4-GFP **(B)** after pull-down reactions using crude protein extracts from 35S:MYC3-HA (M3), 35S:MYC4-GFP (M4) or Col-0 (C) Arabidopsis plants and recombinant MBP or MBP-fused JAZ proteins (top). Coomassie staining shows the input quantity of recombinant proteins used (bottom). Double bands correspond to degradation products of MBP-fused JAZ proteins.

Figure 3. Identification of the domain in MYC TFs interacting with JAZ proteins.

(A) Full-length MYC2 or truncated derivatives were tested for interaction with JAZ1 and JAZ3. Yeast cells co-transformed with pGBKT7-JAZ1 or pGBKT7-JAZ3 (bait) and pGADT7-MYC2 or pGADT7-MYC2-derivatives (prey) were selected and subsequently grown on selective media lacking Ade, His, Leu and Trp (-4), to test protein interactions. The different domains in MYC proteins are represented in scale colors in (A) and (B) colors (blue:bHLH; black: activation domain; grey vertical and horizontal stripes: conserved domains among MYC proteins)

(B) Different N-terminal fragments of MYC2 and MYC3 were assayed for interaction with MYC2-interacting JAZ proteins (JAZ1,2,3,5,6,8,9,10,11 and 12). Yeast cells co-transformed with pGBKT7-MYC2-derivatives or pGBKT7-MYC3-derivatives (bait) and pGADT7-JAZ proteins (prey) were selected and subsequently grown on selective media lacking Ade, His, Leu and Trp (-4), to

test protein interactions. pGBKT7-MYC2 or pGBKT7-MYC3 co-transformation with pGADT7 vector were included as control.

Figure 4. Homo- and heterodimerization among MYC proteins *in planta.*

Immunoblots of co-immunoprecipitated MYC2-HA, MYC3-HA and MYC4-HA (Co-IP; upper panel), immunoprecipitated MYC2-GFP, MYC3-GFP and MYC4-GFP (IP; lower panel) and crude extracts (input HA; middle panel) in transiently expressed proteins in *N. benthamiana* leaves. Immunoprecipitation was performed using anti-GFP matrix, and co-immunoprecipitated proteins were detected using anti-HA antibody. The expression levels of the input HA-fused proteins were assessed by anti-HA of crude extracts (middle panel). The closely related bHLH AtAIB (At2g46510) was used as negative control of interaction and GFP vector as background control of interaction. Asterisks (*) in the lower panel mark the full-length protein band. Protein molecular weight ladder is shown on the left side of each blot.

Figure 5. Identification of MYC2, MYC3 and MYC4 DNA-binding motifs *in vitro*.

(A) Position weight matrix representation of the top scoring 8-mers corresponding to MYC2, MYC3 and MYC4. All three proteins showed highest binding affinity to a canonical G-box (CACGTG).
(B) Enrichment scores (E-scores) of all the possible G-box-containing 8mers for the three MYC proteins tested, showing similar binding preferences of the three proteins for nucleotides at 5´ and 3´ of the G-box 6-mer.
(C) Box-plot of E-scores of G-box variants including both single-site mutations and E-boxes (CANNTG). Boxes represent quartiles 25% to 75%, and black line within represents the median of the distribution (quartile 50%). Bars indicate quartiles 1 to 25% (above) and 75 to 100% (below), and dots denote outliers of the distribution. Boxes in blue correspond to MYC2, green boxes represent MYC3 and yellow ones correspond to data from MYC4.

Figure 6. Tissue expression patterns of MYC3 and MYC4.

Histochemical GUS activity of 8-day-old *A. thaliana* transgenic seedlings (three upper panels) or 4 weeks-old *A. thaliana* plants (lower panels) expressing the GUS reporter gene under the control of the promoter of MYC3 (pMYC3:GUS) or MYC4 (pMYC4:GUS). GUS activity was detected between 3 and 12 hours after staining.

Figure 7. Effect of *myc* mutants on induction of JA-marker genes.

Quantitative RT-PCR of *JAZ10* (1h), *VSP2* (6h) and *PDF1*-2 (24h) expression in mutant and WT plants. The measurements (three technical replicates) represent the ratio between mock and treated (50 μM JA) plants. The level of each gene is relative to ACTIN8 (WT:Col-0, 2:*myc2*, 3:*myc3*, 4:*myc4*,

2;3:*myc2;myc3,* 2;4:*myc2;myc4,* 3;4:*myc3;myc4*, 2;3;4-1:*myc2;myc3;myc4-1,* 2;3;4-2*:myc2;myc3;myc4-2*. Asterisks indicate statistically significant differences compared to Col-0 (Student's t test, * P< 0.05, ** P< 0.01, *** P< 0.001). *2;3;4-1* and *2;3;4-2* represent two different triple mutants obtained from independent crosses but using the same alleles.

Figure 8. Effect of *myc* mutants on root-growth inhibition by JA.

Root-growth inhibition assay of 8-day-old *A. thaliana* seedlings from WT, *coi1-1* and *my2, myc3* and *myc4* single, double and triple mutants grown in 50 µM JA, 0.5 µM coronatine or mock media. Results shown are the mean ± s.d. of measurement from 30 seedlings. Asterisks indicate statistically significant differences between double and triple mutants and *myc2* (One way ANOVA, * P< 0.05, ** P< 0.01, *** P< 0.001). Numbering is as in Figure 7.

Figure 9. Susceptibility of *myc* mutants to a generalist herbivore.

Freshly hatched *S. littoralis* larvae were placed simultaneously on each genotype, and larval weight (mean ± SE) was measured after seven days of feeding. The number of larvae used in each experiment is shown within the bars. Asterisks indicate statistically significant differences compared to Col-0 (Student's t test, * P< 0.05, ** P< 0.01, *** P< 0.001). Similar results were observed in two other independent replicate experiments. The missing additive effect in *myc2;myc3* and *myc2;myc4* double mutants suggest a minor role of MYC2 in insect defence, which is consistent with its low expression in leaves

Figure 10. Resistance of myc mutants to the bacterial hemi-biotrophic pathogen *Pto* DC3000.

(A) Disease symptoms on Col-0 (WT), *myc2 (2)*, *myc3 (3)*, *myc4 (4)*, *myc2;myc3 (2;3)*, *myc2;myc4 (2;4)*, *myc2;myc3;myc4*-1 (*2;3;4-1*),*myc2;myc3;myc4-2* (*2;3;4-2*) and *coi1-1* plants after spray inoculation with *Pto* DC3000 bacteria at 10^8 colony forming units ml^{-1} (cfu/ml). Pictures were taken 3 days after inoculation. Plants show representative symptoms of three independent experiments.

(B) Growth of *Pto* DC3000 on wild-type and mutant *Arabidopsi*s plants 2 days after spray inoculation as in (a). Bacterial counts are expressed as log [colony forming units (cfu) cm^{-2}]. Error bars indicate standard error of the mean (SEM). The results are representative of three independent experiments. Asterisks indicate statistically significant differences compared to Col-0 (Student's t test, * P< 0.05, ** P< 0.01, *** P< 0.001)

Figure 11. Gain-of-function of MYC3 enhance responses to JA.

Root-growth inhibition assay of 8-day-old *A. thaliana* WT seedlings and transgenic lines constitutively expressing *MYC2* (*OEMYC2*), *MYC3* (*OEMYC3*) or *MYC4* (*OEMYC4*) under the control of the CaMV 35S promoter, grown in agar plates supplemented (or not) with 50 µM JA or 0.5 µM coronatine. Results shown are means ± s.d. of measurements from 30 seedlings. Asterisks indicate statistically significant differences compared to Col-0 (Student´s t test P< 0.05, ** P< 0.01, *** P< 0.001).

Table 1. Interactors of MYC and JAZ proteins in TAP-tagging screens.

Left column shows identified proteins that co-purified with transcription factors MYC2-, MYC3- and MYC4-TAP and repressors JAZ3- and JAZ5-TAP expressed in *A. thaliana* cells suspension cultures (PSB-D) as TAP-tagged fusion proteins. Numbers within the table illustrate the number of positive results for each combination prey/bait in four independent TAP experiments, except in the case of JAZ3-TAP were only two experiments were available.

Supplemental Figure Legends

Table S1. MALDI-TOF/TOF-MS identification of MYC2, MYC3, MYC4, JAZ3 and JAZ5 interactors.

Table S2. List of primers used.
Oligonucleotide sequences of primers used within this work to amplify and genotype indicated genes.

Figure S1. **Phylogenetic tree of MYC2-related proteins from Arabidopsis and other plants.**
Phenogram representation of the Maxima Parsimonia method for 17 Arabidopsis, 2 Populus, 2 Rice and 1 Physcomitrella MYC2 bHLH-related proteins. Sequenced alignment was generated using multiple alignment ClustalW algorithm (T-Coffee web server, (http://tcoffee.vital-it.ch) and the tree was created by MEGA4 program. Bootstrap values based on 100 replicates are shown.

Figure S2. Control growth of yeast cells transformed with MYC3 or MYC4 and with JAZ repressors in yeast two-hybrid assays.

Yeast cells co-transformed with pDEST22-MYC2, pDEST22-MYC3, pDEST22-MYC4 or pDEST22-bHLH028 (preys) and pDEST32-JAZ1-12 (baits) were selected and subsequently grown on yeast synthetic drop-out lacking Leu and Trp (-2), as a transformation control.

Figure S3. Alignment of the JAZ-interaction domain of MYC2-related proteins.
(A) Aligned sequences of JAZ interacting domain of MYC2, MYC3, MYC4 and At5g46830 from Arabidopsis and MYC2-closest relatives from Rice (AC060755.9), *Populus* (XM_002329476.1) and *Physcomitrella patens* (XP_001765161.1). Alignment was created with T-Coffee web server

(http://tcoffee.vital-it.ch) using multiple aligment ClustalW algorithm. Residues conserved in all three proteins are labelled by stars, whereas dots indicate residues belonging to the same functional group. The non-conservative change (G-K) in At5g46830 is highlighted with a red square and a red asterisk.
(B) Sequences of the JAZ-interaction domain (JID) of several bHLH proteins of *A. thaliana*: MYC2, MYC3, MYC4,At5g46830 (BHLH028), At5g41315 (GL3), At1g63650 (EGL3), At4g09820 (TT8), At1g01260 (BHLH013), At2g46510 (AtAIB) and At4g16430 (BHLH003). Sequence alignment was generated as in (A).

Figure S4. MYC3 and MYC4 are nulear-localized bHLH.

Confocal microscope visualization of nuclear-localized MYC4-GFP and MYC3-GFP fusion proteins in the roots of 35S:MYC4-GFP (upper panels) and 35S:MYC3-GFP (lower panels) transgenic Arabidopsis seedlings.

Figure S5. **JA dependent induction of *MYC3* and *MYC4* gene expression.**

Quantitative RT-PCR analysis of *MYC2, MYC3* and *MYC4* JA dependent induction. 8-days-old WT (Col0) seedlings were assayed for the expression of these three genes in a time-course experiment (1, 6 and 24 hours) in response to 50 µM JA. The measurements (three technical replicates) represent the ratio between mock and treated (50 µM JA) plants and the level of each gene is relative to ACTIN8.

Figure S6. Expression of MYC genes in *myc* mutant backgrounds.

MYC2, MYC3 and *MYC4* expression was analyzed in WT (Col-0) and mutant backgrounds *myc2, myc3* and *myc4.* Quantitative RT-PCR measurements were carried out in 8-days-old seedlings growing in control media. Measurements (three technical replicates) represent the level of each gene relative to ACTIN8 normalized to WT (Col-0) values.

Figure S7. MYC2, MYC3 and MYC4 tissue expression patterns.
Genevestigator array data of MYC2, MYC3 and MYC4 expression levels in different *Arabidopsis thaliana* tissues and developmental stages.

Table 1

	MYC4	MYC3	MYC2	JAZ3	JAZ5
JAZ2	3	4	2		2
JAZ3				2	
JAZ5					4
JAZ11	4		1		
JAZ12	4	4	3		4
MYC4	4	2			
MYC3	4	4	3	1	2
MYC2			3		
NINJA	4	4	3	2	4
TPR1/TOPLESS				1	2
TPR2					1
TPR3					1

Table 1. Interactors of MYC and JAZ proteins in TAP-tagging screens.
Left column shows identified proteins that co-purified with transcription factors MYC2-, MYC3- and MYC4-TAP and repressors JAZ3- and JAZ5-TAP expressed in *A. thaliana* cells suspension cultures (PSB-D) as TAP-tagged fusion proteins. Numbers within the table illustrate the number of positive results for each combination prey/bait in four independent TAP experiments, except in the case of JAZ3-TAP were only two experiments were available.

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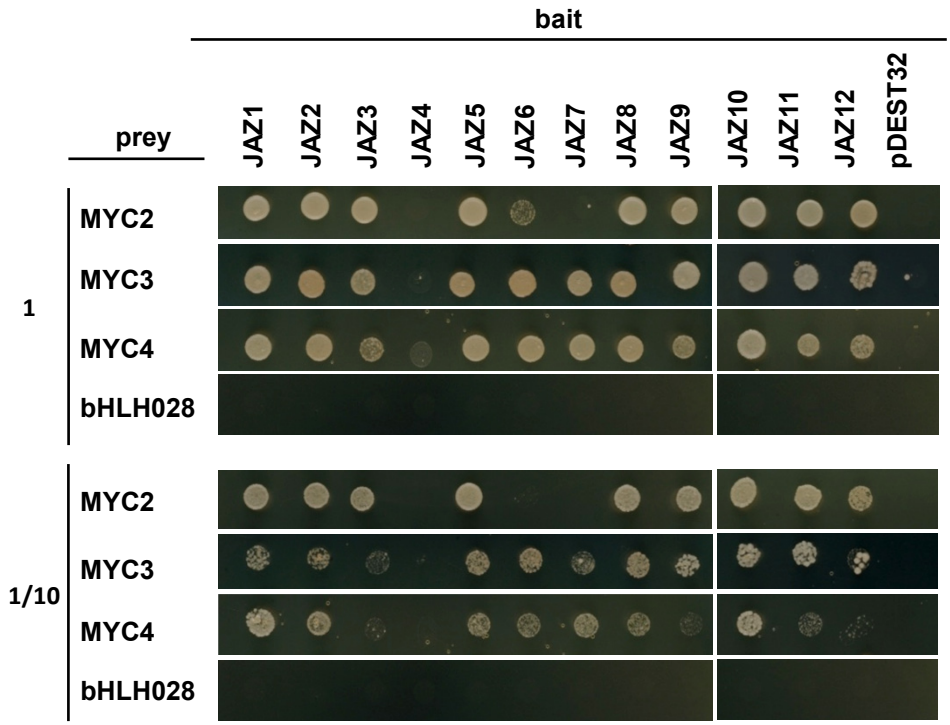


Figure 1. MYC3 and MYC4 interact with JAZ repressors in yeast two-hybrid assays.

Yeast cells co-transformed with pDEST22-MYC2, pDEST22-MYC3 , pDEST22-MYC4 or pDEST22-bHLH028 (preys) and pDEST32-JAZ1-12 (baits) were selected and subsequently grown on yeast synthetic drop-out lacking Leu and Trp (-2), as a transformation control (shown in Supplemental Figure S2), or on selective media lacking Ade, His, Leu and Trp (-4), to test protein interactions (this figure). The lower panel show 1/10 dilution yeast growth in – 4 selective media. pDEST22-MYC2, pDEST22-MYC3, pDEST22-MYC4 and pDEST22-bHLH028 co-transformation with pDEST32 vector were included as controls.

Figure 1

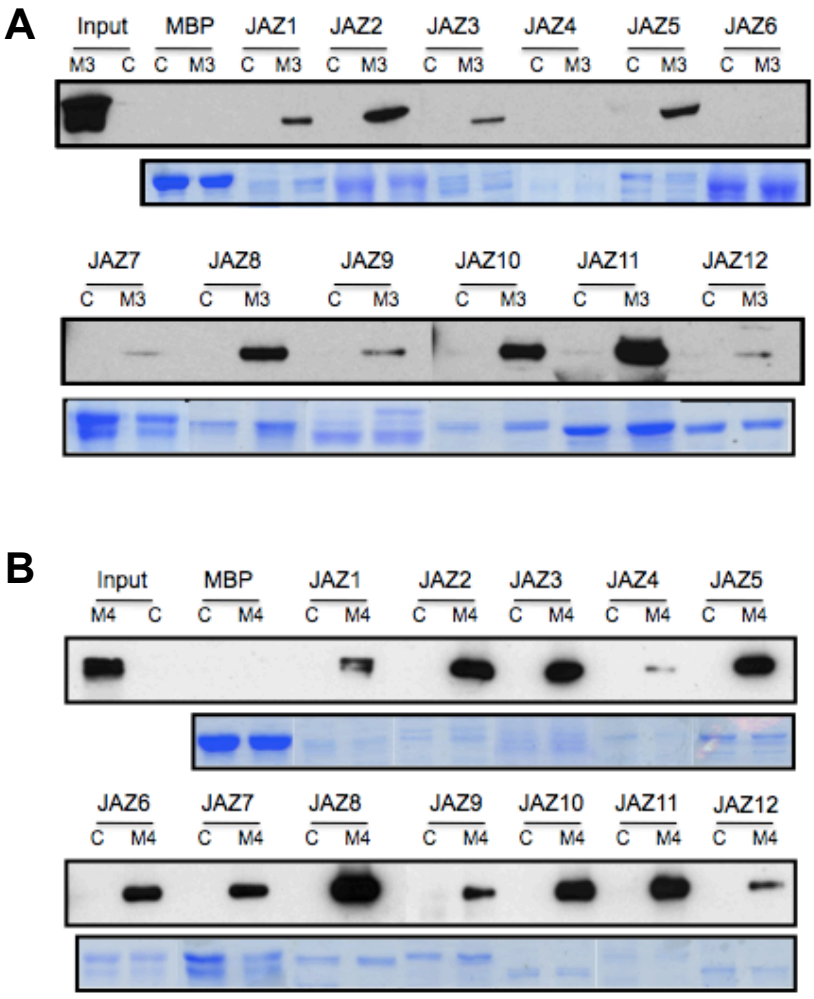


Figure 2. MYC3 and MYC4 interact with JAZ repressors in Pull-down experiments. Immunoblots with anti-HA antibody of recovered MYC3-HA **(A)** or with anti-GFP antibody of recovered MYC4-GFP **(B)** after pull-down reactions using crude protein extracts from 35S:MYC3-HA (M3), 35S:MYC4-GFP (M4) or Col-0 (C) Arabidopsis plants and recombinant MBP or MBP-fused JAZ proteins (top). Coomassie staining shows the input quantity of recombinant proteins used (bottom). Double bands correspond to degradation products of MBP-fused JAZ proteins.

Figure 2

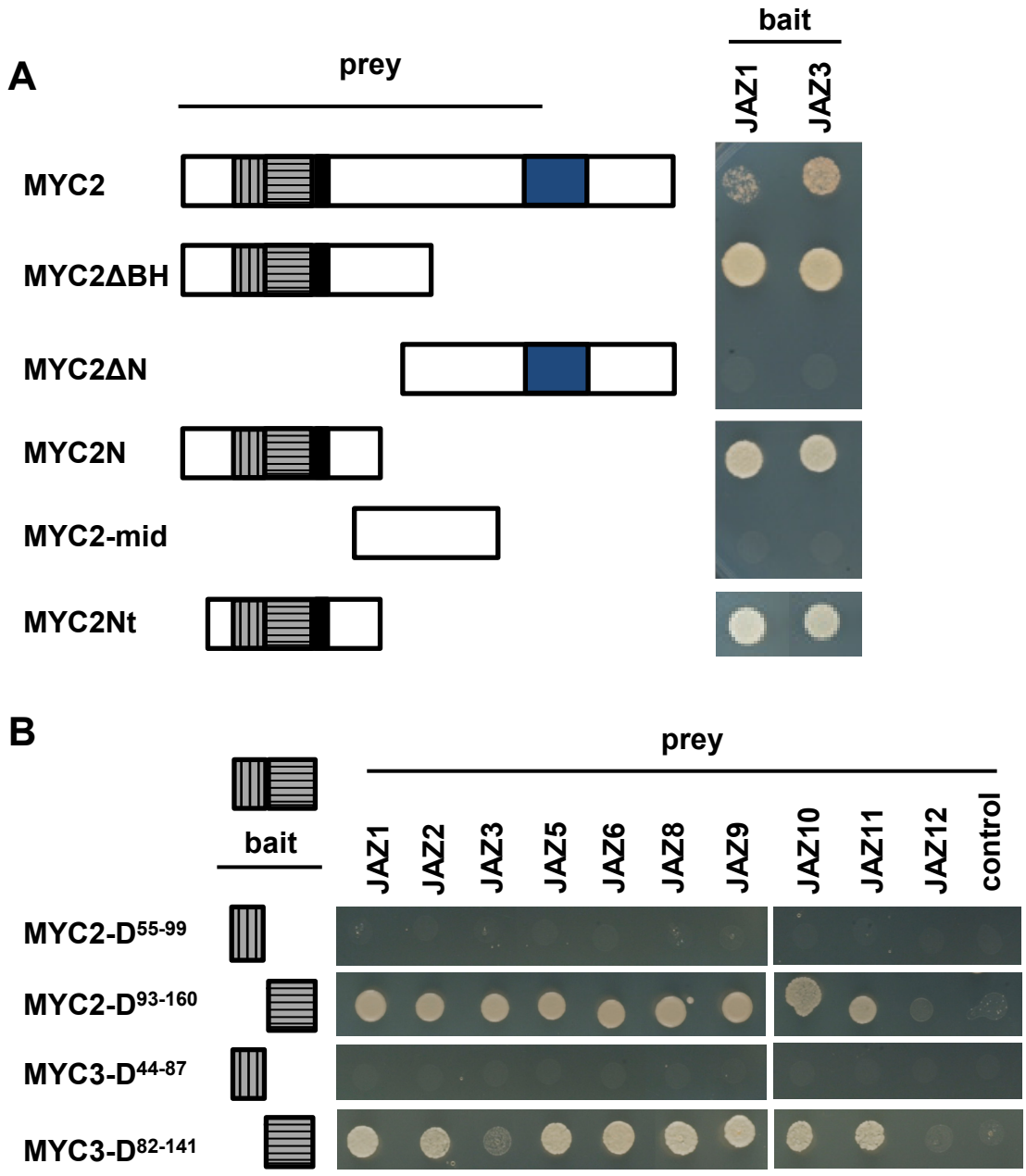


Figure 3. Identification of the domain in MYC TFs interacting with JAZ proteins.
(A) Full-length MYC2 or truncated derivatives were tested for interaction with JAZ1 and JAZ3. Yeast cells co-transformed with pGBKT7-JAZ1 or pGGBT7-JAZ3 (bait) and pGADT7-MYC2 or pGADT7-MYC2-derivatives (prey) were selected and subsequently grown on selective media lacking Ade, His, Leu and Trp (-4; shown in the figure) to test protein interactions. The different domains in MYC proteins are represented in scale and colors (blue:bHLH; black: activation domain; grey vertical and horizontal stripes: conserved domains among MYC proteins).
(B) Different N-terminal fragments of MYC2 and MYC3 were assayed for interaction with MYC2-interacting JAZ proteins (JAZ1,2,3,5,6,8,9,10,11 and 12). Yeast cells co-transformed with pGBKT7-MYC2-derivatives or pGBKT7-MYC3-derivatives (bait) and pGADT7-JAZ proteins (prey) were selected and subsequently grown on selective media lacking Ade, His, Leu and Trp (-4; shown in the figure), to test protein interactions. pGBKT7-MYC2 and pGBKT7-MYC3 co-transformation with pGADT7 vector were included as controls.

Figure 3

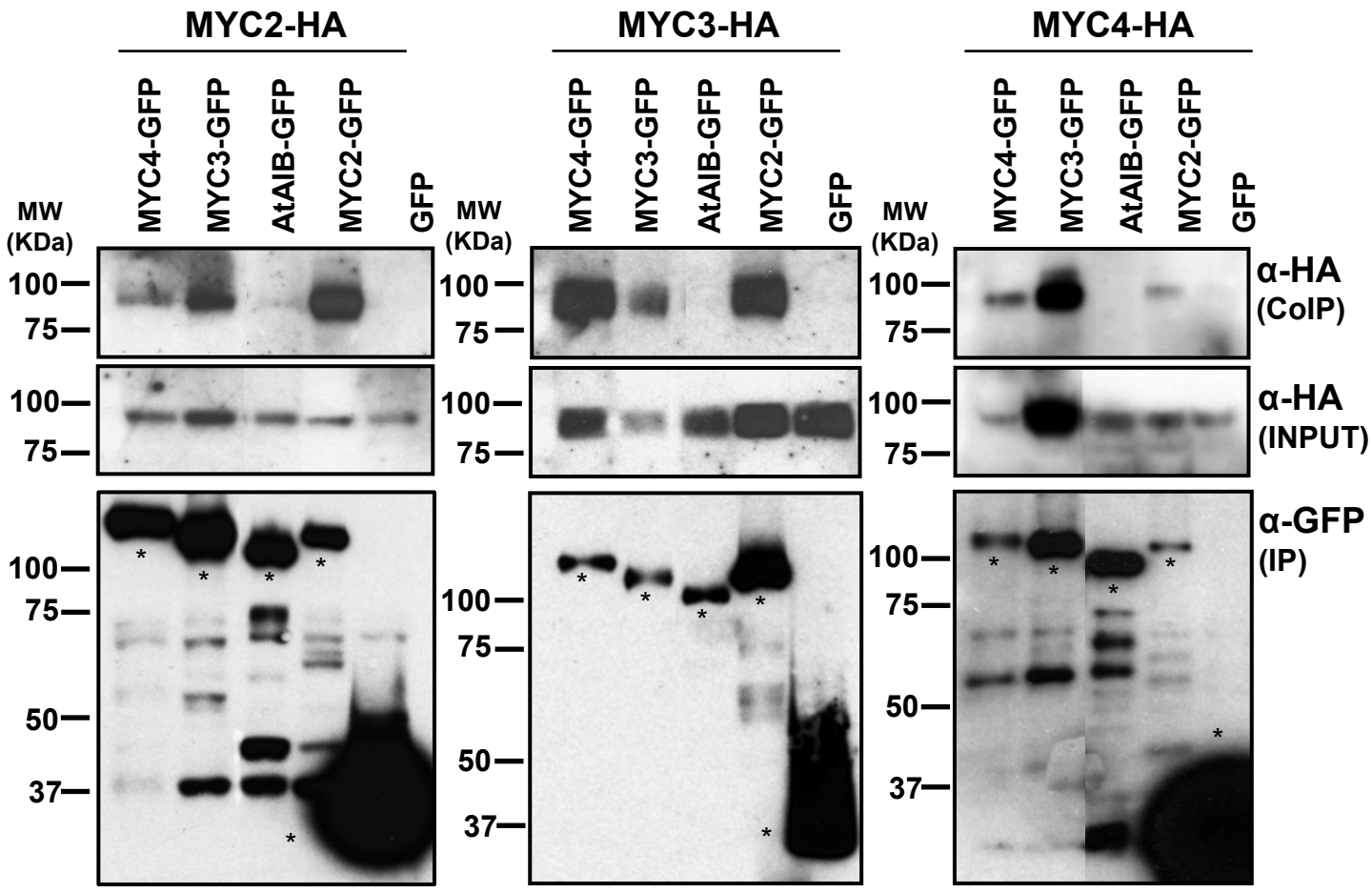


Figure 4. Homo- and heterodimerization among MYC proteins *in planta.*
Immunoblots of co-immunoprecipitated MYC2-HA, MYC3-HA and MYC4-HA (Co-IP; upper panel), immunoprecipitated MYC2-GFP, MYC3-GFP and MYC4-GFP (IP; lower panel) and crude extracts (input HA; middle panel) in transiently expressed proteins in *N. benthamiana* leaves. Immunoprecipitation was performed using anti-GFP matrix, and co-immunoprecipitated proteins were detected using anti-HA antibody. The expression levels of the input HA-fused proteins were assessed by anti-HA of crude extracts (middle panel). The closely related bHLH AtAIB (At2g46510) was used as negative control of interaction and GFP vector as background control of interaction. Asterisks (*) in the lower panel mark the full-length protein band. Protein molecular weight ladder is shown in the left side of each blot.

Figure 4

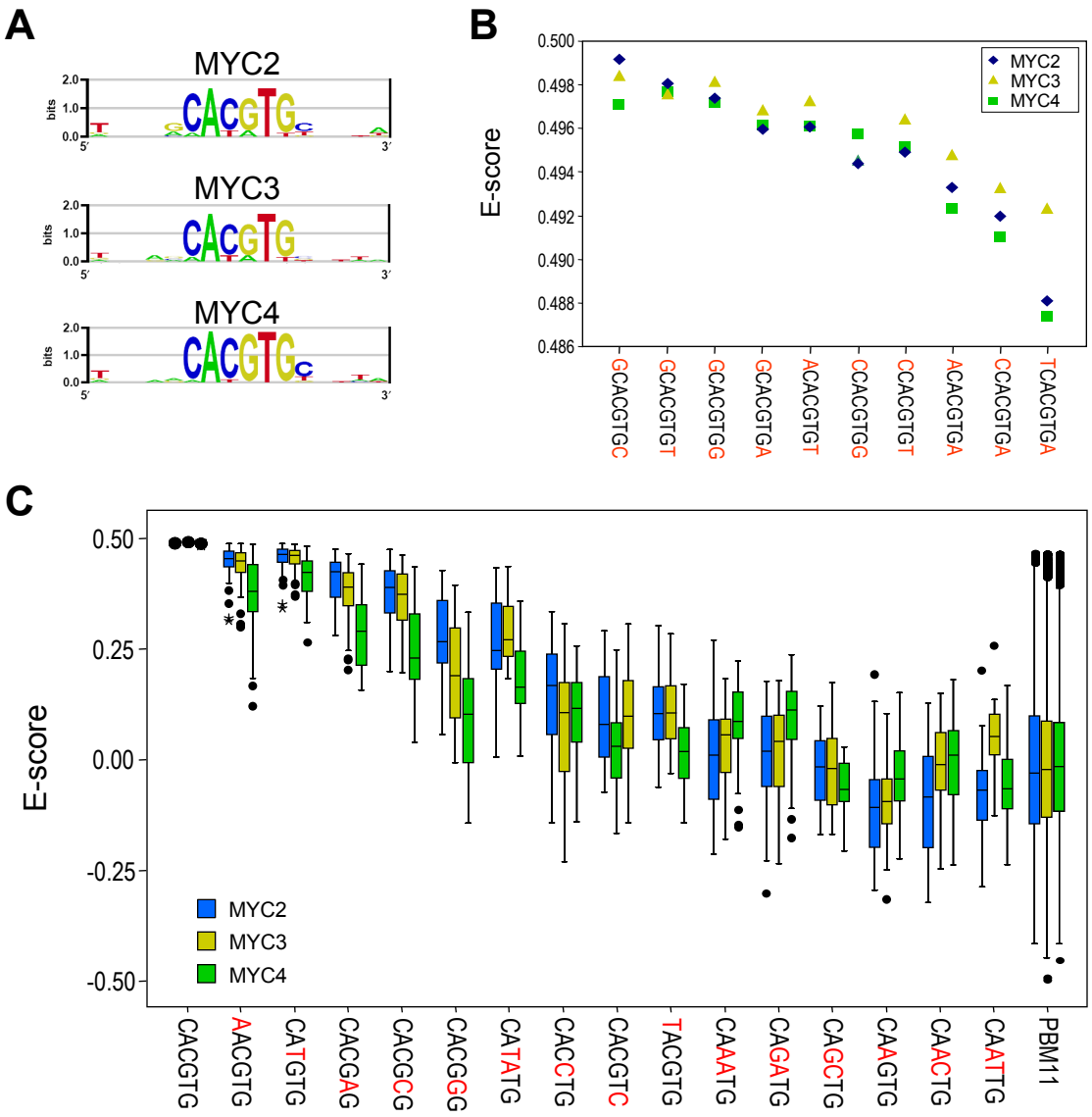


Figure 5. Identification of MYC2, MYC3 and MYC4 DNA-binding motifs *in vitro*.
(A) Position weight matrix representation of the top scoring 8-mers corresponding to MYC2, MYC3 and MYC4. All three proteins showed highest binding affinity to a canonical G-box (CACGTG).
(B) Enrichment scores (E-scores) of all the possible G-box-containing 8mers for the three MYC proteins tested, showing similar binding preferences of the three proteins for nucleotides at 5´ and 3´ of the G-box 6-mer.
(C) Box-plot of E-scores of G-box variants including both single-site mutations and E-boxes (CANNTG). Boxes represent quartiles 25% to 75%, and black line within represents the median of the distribution (quartile 50%). Bars indicate quartiles 1 to 25% (above) and 75 to 100% (below), and dots denote outliers of the distribution. Boxes in blue correspond to MYC2, green boxes represent MYC3 and yellow ones correspond to data from MYC4.

Figure 5

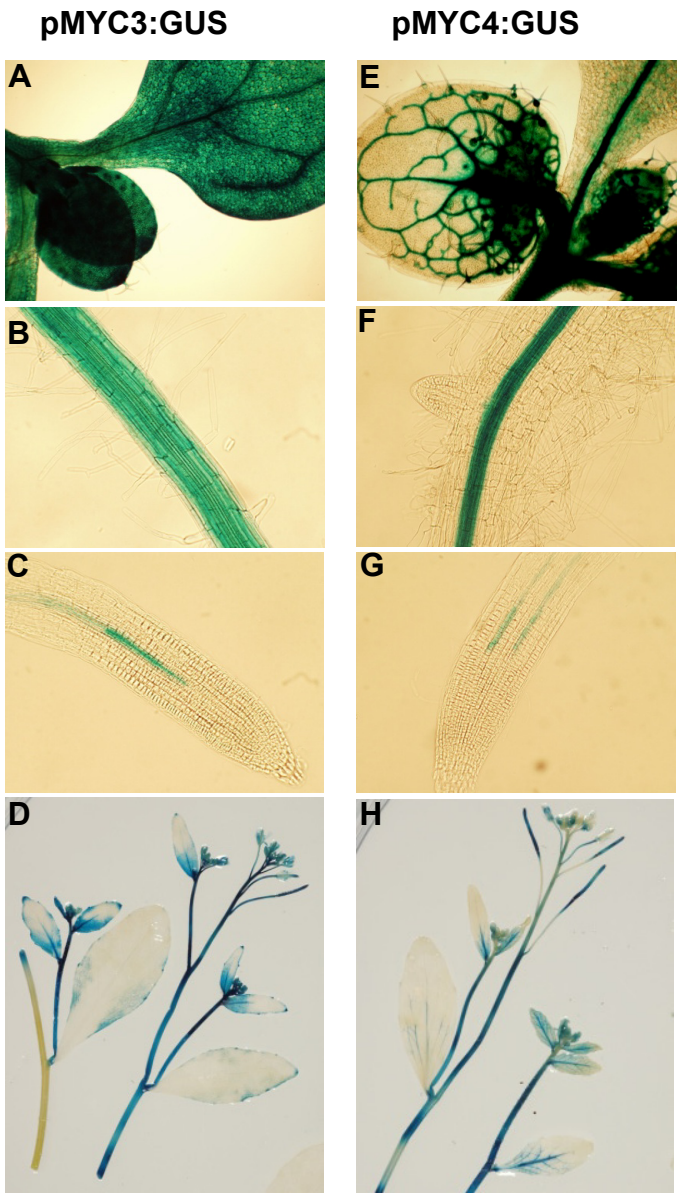


Figure 6. Tissue expression patterns of MYC3 and MYC4.

Histochemical GUS activity of 8-day-old *A. thaliana* transgenic seedlings in upper panels, **(A)**, **(B)**, **(C)**, **(E)**, **(F)** and **(G)** or 4 weeks-old *A. thaliana* plants in lower panels, **(D)** and **(H)** expressing the GUS reporter gene under the control of the promoter of MYC3 (pMYC3:GUS) or MYC4 (pMYC4:GUS). GUS activity was detected between 3 and 12 hours after staining.

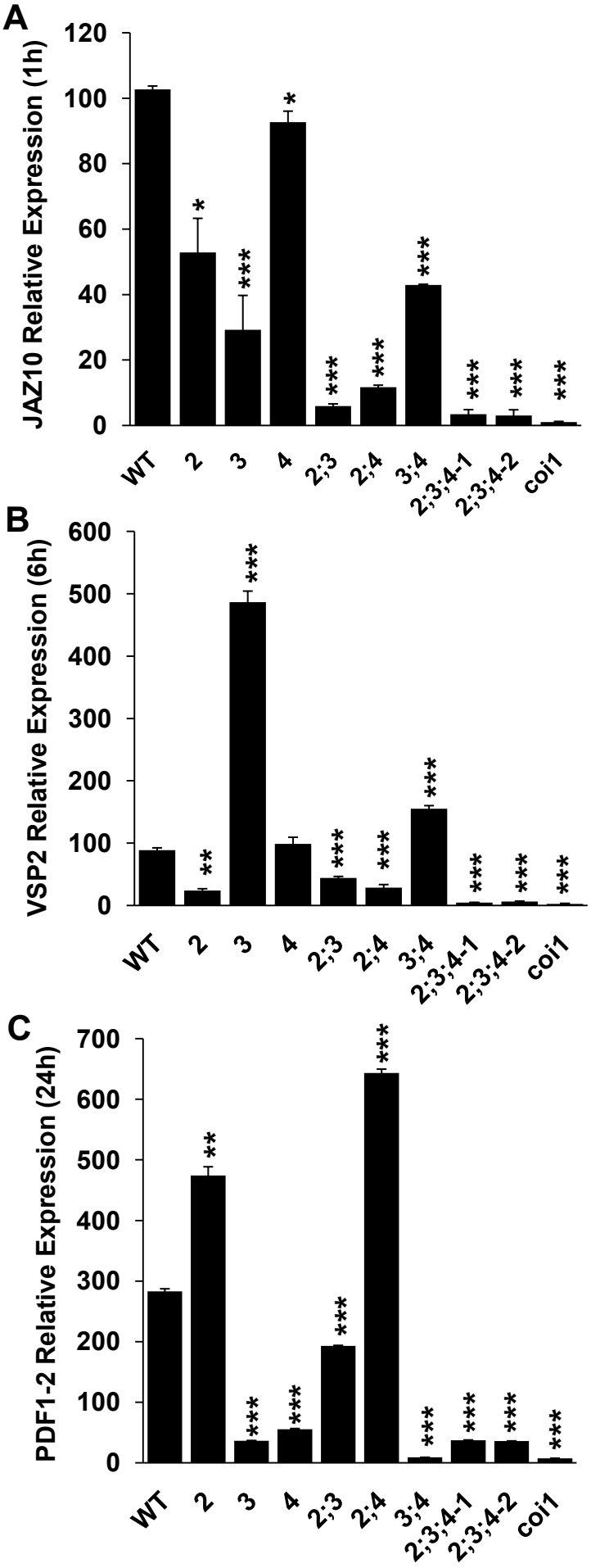


Figure 7. Effect of *myc* mutants on induction of JA-marker genes.

Quantitative RT-PCR of **(A)** *JAZ10* (1h), **(B)** *VSP2* (6h) and **(C)** *PDF1*-2 (24h) expression in mutant and WT plants. The measurements (three technical replicates) represent the ratio between mock and treated (50 µM JA) plants. The level of each gene is relative to ACTIN8 (WT: Col0, 2: *myc2*, 3: *myc3*, 4: *myc4*, 2;3: *myc2;myc3,* 2;4: *myc2;myc4,* 3;4: *myc3;myc4*, 2;3;4-1: *myc2;myc3;myc4-1,* 2;3;4-2: *myc2;myc3;myc4-2*. Asterisks indicate statistically significant differences compared to Col-0 (Student's t test, * P< 0.05, ** P< 0.01, *** P< 0.001). 2;3;4-1 and 2;3;4-2 represent two different triple mutants obtained from independent crosses but using the same alleles.

Figure 7

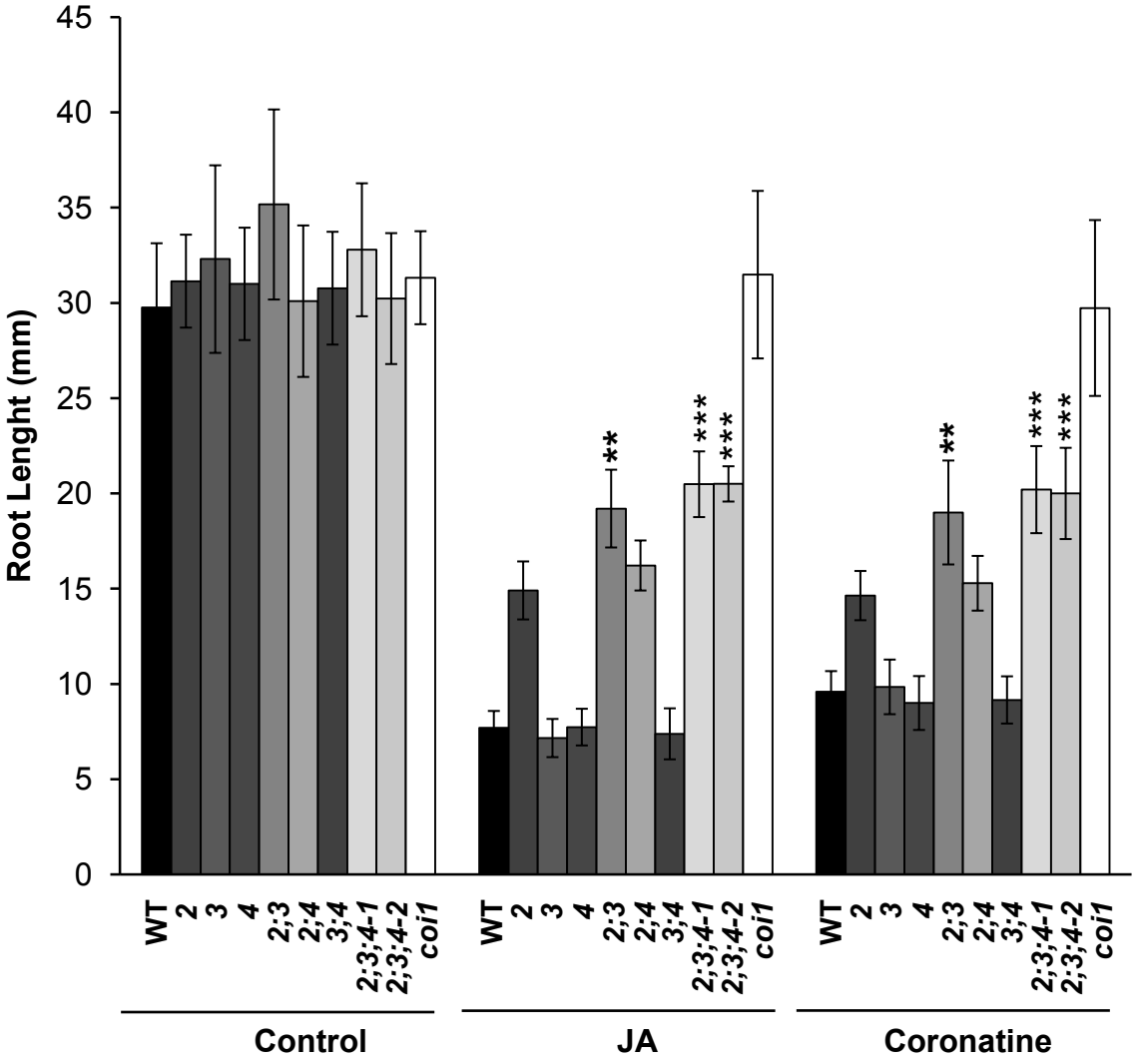


Figure 8. Effect of *myc* mutants on root-growth inhibition by JA.
Root-growth inhibition assay of 8-day-old *A. thaliana* seedlings from WT, *coi1-1* and *my2, myc3* and *myc4* single, double and triple mutants grown in 50 µM JA, 0.5 µM coronatine or mock media. Results shown are the mean ± s.d. of measurement from 30 seedlings. Asterisks indicate statistically significant differences between double and triple mutants and *myc2* (One way ANOVA, * P< 0.05, ** P< 0.01, *** P< 0.001). Numbering is as in Figure 7.

Figure 8

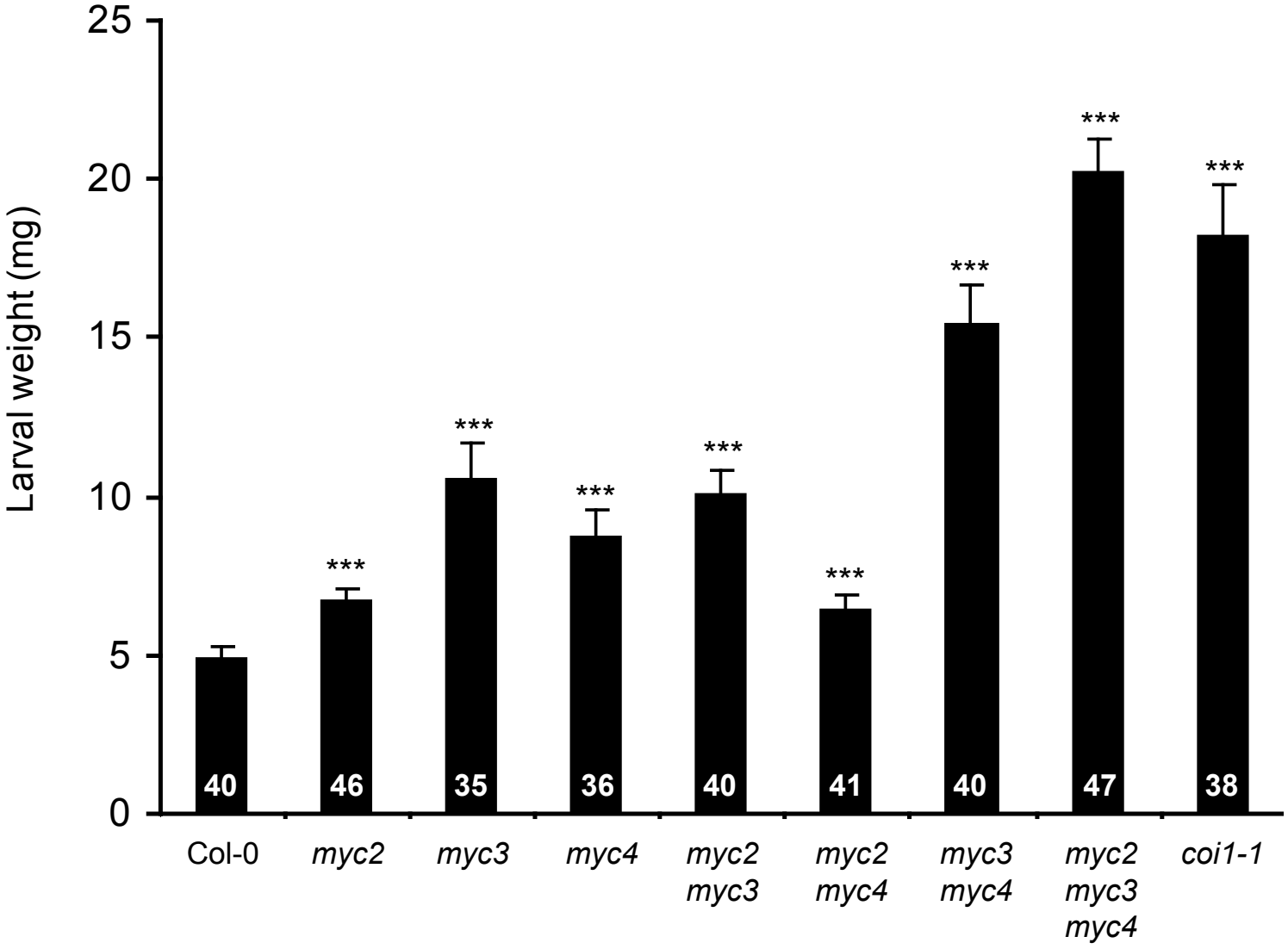


Figure 9. Susceptibility of *myc* mutants to a generalist herbivore.
Freshly hatched *S. littoralis* larvae were placed simultaneously on each genotype, and larval weight (mean ± SE) was measured after seven days of feeding. The number of larvae used in each experiment is shown within the bars. Asterisks indicate statistically significant differences compared to Col-0 (Student's t test, * P< 0.05, ** P< 0.01, *** P< 0.001). Similar results were observed in two other independent replicate experiments. The missing additive effect in *myc2;myc3* and *myc2;myc4* double mutants suggest a minor role of MYC2 in insect defence, which is consistent with its low expression in leaves.

Figure 9

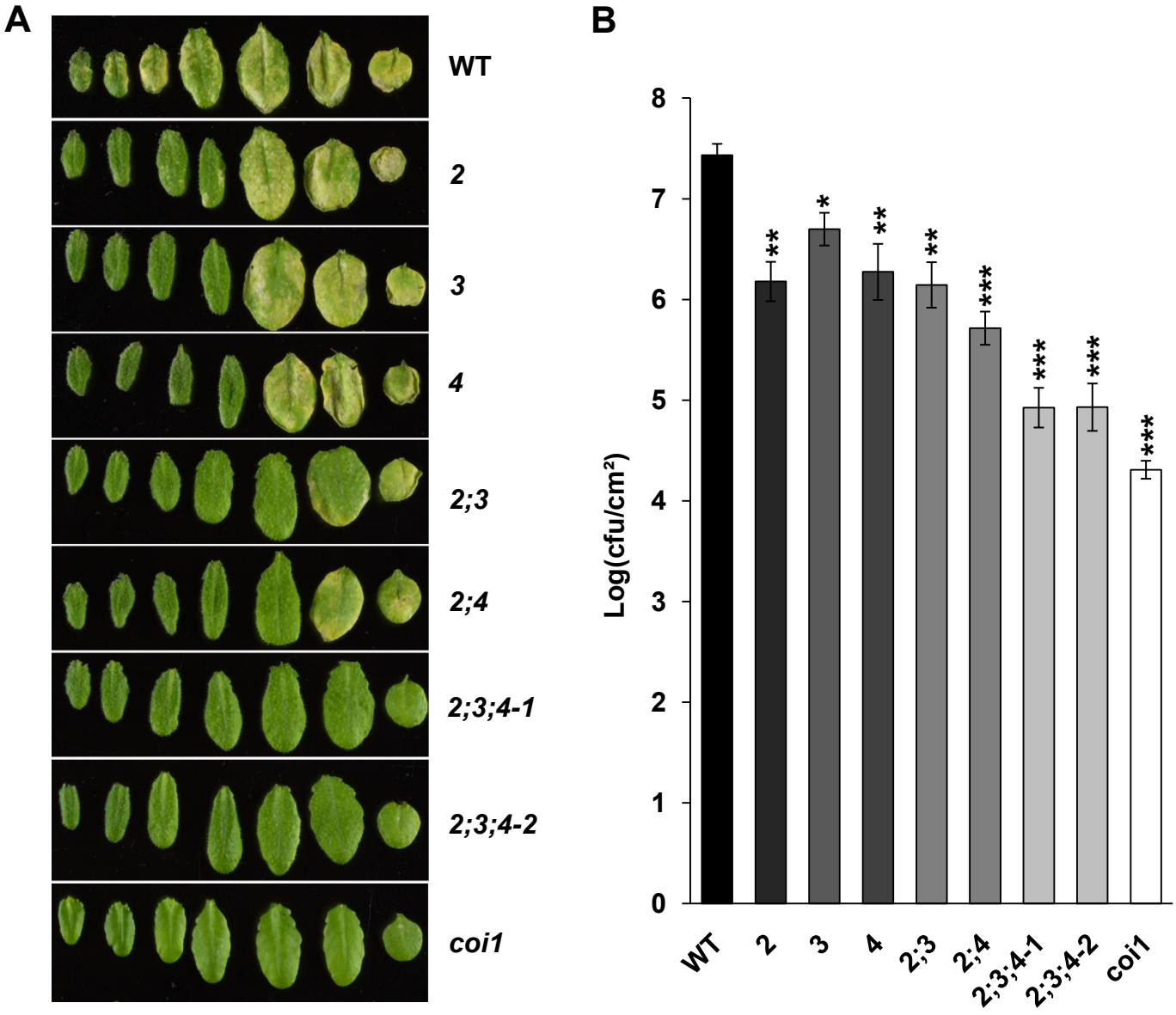


Figure 10. Resistance of myc mutants to the bacterial hemi-biotrophic pathogen *Pto* DC3000.

(A) Disease symptoms on Col-0 (WT), *myc2 (2)*, *myc3 (3)*, *myc4 (4)*, *myc2;myc3 (2;3)*, *myc2;myc4 (2;4)*, *myc2;myc3;myc4*-1 (*2;3;4-1*),*myc2;myc3;myc4*-2 (*2;3;4-2*) and *coi1-1* plants after spray inoculation with *Pto* DC3000 bacteria at 10^8 colony forming units ml^{-1} (cfu/ml). Pictures were taken 3 days after inoculation. Plants show representative symptoms of three independent experiments.

(B) Growth of *Pto* DC3000 on wild-type and mutant *Arabidopsi*s plants 2 days after spray inoculation as in (a). Bacterial counts are expressed as log [colony forming units (cfu) cm^{-2}]. Error bars indicate standard error of the mean (SEM). The results are representative of three independent experiments. Asterisks indicate statistically significant differences compared to Col-0 (Student's t test, * P< 0.05, ** P< 0.01, *** P< 0.001).

Figure 10

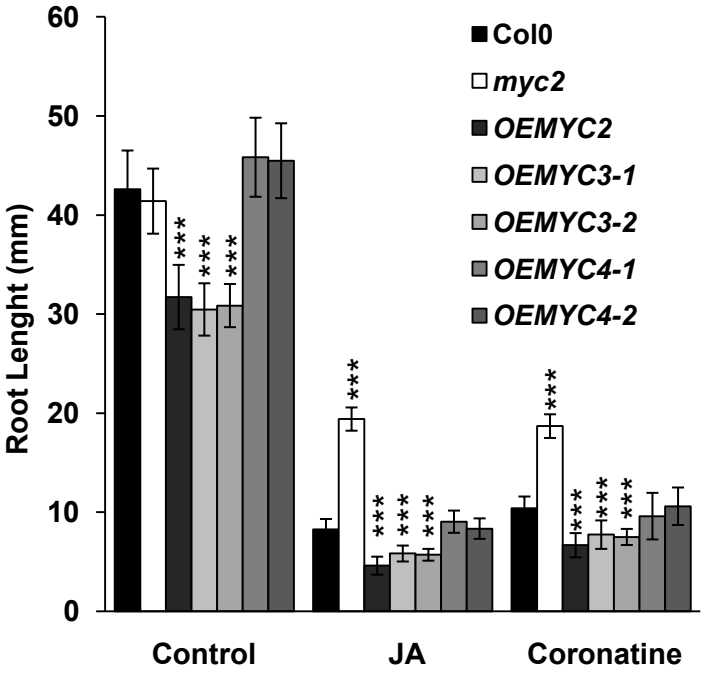


Figure 11. Gain-of-function of MYC3 enhance responses to JA.

Root-growth inhibition assay of 8-day-old *A. thaliana* WT seedlings and transgenic lines constitutively expressing *MYC2* (*OEMYC2*), *MYC3* (*OEMYC3*) or *MYC4* (*OEMYC4*) under the control of the CaMV 35S promoter, grown in agar plates supplemented (or not) with 50 µM JA or 0.5 µM coronatine. Results shown are means ± s.d. of measurements from 30 seedlings. Asterisks indicate statistically significant differences compared to Col-0 (Student´s t test * P< 0.05, ** P< 0.01, *** P< 0.001).

Figure 11

	MYC4	MYC3	MYC2	JAZ3	JAZ5
JAZ2	3	4	2		2
JAZ3				2	
JAZ5					4
JAZ11	4		1		
JAZ12	4	4	3		4
MYC4	4	2			
MYC3	4	4	3	1	2
MYC2			3		
NINJA	4	4	3	2	4
TPR1/TOPLESS				1	2
TPR2					1
TPR3					1

Table 1. Interactors of MYC and JAZ proteins in TAP-tagging screens.
Left column shows identified proteins that co-purified with transcription factors MYC2-, MYC3- and MYC4-TAP and repressors JAZ3- and JAZ5-TAP expressed in *A. thaliana* cells suspension cultures (PSB-D) in TAP-tagging experiments. Numbers within the table illustrate the number of positive results for each combination prey/bait in four independent TAP experiments, except in the case of JAZ3-TAP were only two experiments were available.

Supplemental Table 1 **Table S1. MALDI-TOF/TOF-MS identification of MYC2, MYC3, MYC4, JAZ3 and JAZ5 interactors.**

Bait	Prey				PMF data					MSMS data												
Bait	Prey Locus	Prey Name	# Found/ # exp	Database version	Protein Score	Expect	RMS error (ppm)	Sequence coverage %	Unique Peptides	Total Ion Score	Peptide Number	Start	End	Observed	Mr(Exp)	Mr(Calc)	Delta	Miss	Ions Score	Expect	Peptide	Variable Modification
MYC2	AT1G32640	MYC2 (BAIT)	3/3	TAIR8	725	1,00E-68	5	27	15	621	1	341	347	885,42	884,41	884,41	0,00	0	43	5,70E-04	K.FNNTFSR.E	
											2	608	615	1050,56	1049,55	1049,55	0,00	0	46	7,00E-04	R.IYTQEQLR.A	
											3	*552*	*560*	*1090,57*	*1089,56*	*1089,56*	*0,00*	*0*	*25*	*1,10E-01*	*K.IIGWDAMIR.V*	*Oxidation (M)*
											4	363	375	1421,70	1420,69	1420,69	0,00	1	79	3,60E-07	R.SGEILNFGDEGKR.S	
											5	513	525	1498,82	1497,81	1497,82	0,00	1	70	1,40E-06	K.NQLEEVKLELAGR.K	
											6	484	498	1590,87	1589,86	1589,87	-0,01	0	108	2,40E-10	K.ASLLGDAIAYINELK.S	
											7	376	395	2185,98	2184,97	2184,97	0,00	1	101	6,30E-10	R.SSGNPDPSSYSGGQTQFENKR.K	
											8	186	208	2356,06	2355,06	2355,05	0,00	0	68	1,70E-06	K.AFATGNAVWVSGSDQLSGSGCER.A	
											9	211	239	2983,49	2982,48	2982,47	0,02	0	83	5,70E-08	K.QGGVFGMHTIACIPSANGVVEVGSTEPIR.Q	
MYC2	AT5G46760	MYC3	3/3	TAIR8	404	1,30E-36	6	25	11	338	1	192	201	1034,56	1033,56	1033,56	0,00	0	63	1,21E-05	R.AGQGQIYGLK.T	
											2	324	333	1091,60	1090,59	1090,60	-0,01	0	66	6,10E-06	K.DLTFQGGLLK.S	
											3	*518*	*526*	*1102,61*	*1101,60*	*1101,60*	*0,00*	*0*	*26*	*6,50E-02*	*K.IIGWDVMIR.V*	
											4	*518*	*526*	*1118,60*	*1117,59*	*1117,60*	*0,00*	*0*	*24*	*1,09E-01*	*K.IIGWDVMIR.V*	*Oxidation (M)*
											5	570	581	1451,67	1450,66	1450,67	-0,01	0	34	7,40E-03	K.MGSQFFNHDQLK.V	
											6	570	581	1467,66	1466,66	1466,66	-0,01	0	35	4,51E-03	K.MGSQFFNHDQLK.V	Oxidation (M)
											7	447	461	1606,86	1605,86	1605,86	0,00	0	69	1,94E-06	K.ASLLGDAISYINELK.S	
											8	*355*	*370*	*1712,79*	*1711,78*	*1711,78*	*0,00*	*0*	*23*	*8,08E-02*	*K.GSNNDEGMLSFSTVVR.S*	
											9	175	191	1749,85	1748,85	1748,85	0,00	0	55	6,08E-05	R.VIWLSGSGALTGSGCER.A	
MYC2	AT4G28910	NINJA	3/3	TAIR8	261	2,60E-22	3	37	12	172	1	372	379	896,48	895,48	895,48	0,00	0	40	2,43E-03	R.TISGVTYR.Y	
											2	276	292	1618,82	1617,81	1617,81	0,00	0	85	6,61E-08	K.DGSGGIVALSQSPFAGR.V	
											3	*73*	*89*	*1908,87*	*1907,87*	*1907,86*	*0,00*	*0*	*28*	*1,97E-02*	*R.SDSGQQPPQNFFNDLSK.A*	
											4	*179*	*197*	*2124,11*	*2123,10*	*2123,10*	*0,00*	*0*	*20*	*1,74E-01*	*K.EVVRPPTDTNIVDNLTGQR.R*	
MYC2	AT5G20900	JAZ12	3/3	TAIR8	240	3,30E-20	1	39	7	181	1	161	169	1068,50	1067,49	1067,49	0,00	0	34	7,62E-03	K.NPYPTSDFK.K	
											2	161	170	1196,59	1195,58	1195,59	0,00	1	49	2,91E-04	K.NPYPTSDFKK.T	
											3	92	106	1529,79	1528,78	1528,78	0,00	0	63	1,11E-05	K.NSTSISPVSSPALNR.A	
											4	*171*	*187*	*1804,90*	*1803,89*	*1803,89*	*0,00*	*1*	*25*	*6,52E-02*	*K.TDVPTGNVSIKEEFPTA.*	
											5	*107*	*133*	*2864,40*	*2863,39*	*2863,40*	*0,00*	*0*	*10*	*1,17E+00*	*R.APSFSSTSNVASPAAQPFPIQPISFCR.S*	
MYC2	AT1G74950	JAZ2	2/3	TAIR8	124	1,30E-08	8	41	6	77	*1*	*15*	*24*	*1211,58*	*1210,58*	*1210,58*	*0,00*	*0*	*25*	*6,83E-02*	*R.KPSFSQTCTR.L*	
											2	110	127	1937,01	1936,00	1936,01	0,00	0	52	1,08E-04	K.SVKPESQSAPLTIFYGGR.V	
MYC2	AT3G43440	JAZ11	1/3	TAIR8	60	3,40E-02	0	12	2	51	1	80	90	1168,64	1167,64	1167,64	0,00	0	35	4,83E-03	K.NVTGINPALNR.A	
											2	*37*	*55*	*2039,97*	*2038,96*	*2038,96*	*0,00*	*0*	*16*	*4,65E-01*	*R.STEPDASTQLTIIFGGSCR.V*	
MYC3	AT5G46760	MYC3 (BAIT)	4/4	TAIR8	799	4,10E-76	3	27	14	704	1	192	201	1034,56	1033,55	1033,56	0,00	0	71	2,10E-06	R.AGQGQIYGLK.T	
											2	324	333	1091,61	1090,60	1090,60	0,00	0	77	4,60E-07	K.DLTFQGGLLK.S	
											3	518	526	1102,60	1101,59	1101,60	-0,01	0	54	1,20E-04	K.IIGWDVMIR.V	
											4	*518*	*526*	*1118,60*	*1117,59*	*1117,60*	*0,00*	*0*	*23*	*1,50E-01*	*K.IIGWDVMIR.V*	*Oxidation (M)*
											5	570	581	1451,67	1450,66	1450,67	0,00	0	72	1,10E-06	K.MGSQFFNHDQLK.V	
											6	570	581	1467,66	1466,66	1466,66	0,00	0	42	9,20E-04	K.MGSQFFNHDQLK.V	Oxidation (M)
											7	447	461	1606,87	1605,86	1605,86	0,00	0	98	3,00E-09	K.ASLLGDAISYINELK.S	
											8	355	370	1712,79	1711,78	1711,78	0,00	0	119	1,90E-11	K.GSNNDEGMLSFSTVVR.S	
											9	355	370	1728,78	1727,78	1727,78	0,00	0	84	5,50E-08	K.GSNNDEGMLSFSTVVR.S	Oxidation (M)
											10	175	191	1749,86	1748,85	1748,85	0,00	0	139	2,40E-13	R.VIWLSGSGALTGSGCER.A	
											11	405	421	1945,98	1944,98	1944,98	0,00	1	75	6,70E-07	R.KPANGREEPLNHVEAER.Q	
MYC3	AT5G20900	JAZ12	4/4	TAIR8	534	1,30E-49	1	53	8	460	1	161	169	1068,50	1067,49	1067,49	0,00	0	45	5,50E-04	K.NPYPTSDFK.K	
											2	161	170	1196,59	1195,59	1195,59	0,00	1	60	2,20E-05	K.NPYPTSDFKK.T	
											3	92	106	1529,79	1528,78	1528,78	0,00	0	76	5,40E-07	K.NSTSISPVSSPALNR.A	
											4	171	187	1804,89	1803,89	1803,89	0,00	1	68	3,00E-06	K.TDVPTGNVSIKEEFPTA.-	
											5	10	36	2407,07	2406,07	2406,06	0,01	0	142	5,40E-14	R.ASVEGGCGVADGDGGAAEIGGTGSVEK.S	
											6	107	133	2864,41	2863,40	2863,40	0,01	0	69	1,60E-06	R.APSFSSTSNVASPAAQPFPIQPISFCR.S	
MYC3	AT4G28910	NINJA	4/4	TAIR9	409	4,20E-37	3	46	14	291	1	276	292	1618,82	1617,81	1617,81	0,57	0	124	8,80E-12	K.DGSGGIVALSQSPFAGR.V	
											2	73	89	1908,86	1907,85	1907,87	-5,27	0	66	2,40E-06	R.SDSGQQPPQNFFNDLSK.A	
											3	*387*	*403*	*2031,89*	*2030,88*	*2030,88*	*1,14*	*0*	*17*	*1,40E-01*	*K.IVCACHGSHMSPEEFVR.H*	*Oxidation (M)*
											4	179	197	2124,10	2123,09	2123,10	-3,05	0	67	3,20E-06	K.EVVRPPTDTNIVDNLTGQR.R	
											5	*349*	*371*	*2295,13*	*2294,13*	*2294,13*	*-2,04*	*0*	*17*	*3,50E-01*	*K.FGGSGARPNLPWVSTTGSGPHGR.T*	
MYC3	AT1G74950	JAZ2	4/4	TAIR8	388	5,20E-35	5	51	8	319	*1*	*15*	*24*	*1211,58*	*1210,58*	*1210,58*	*0,00*	*0*	*19*	*2,50E-01*	*R.KPSFSQTCTR.L*	
											2	128	138	1303,58	1302,58	1302,58	0,00	0	54	2,60E-05	R.VMVFDDFSAEK.A	Oxidation (M)
											3	110	127	1937,01	1936,00	1936,01	0,00	0	106	4,30E-10	K.SVKPESQSAPLTIFYGGR.V	
											4	*33*	*53*	*2170,99*	*2169,98*	*2169,98*	*0,00*	*0*	*15*	*3,60E-01*	*K.GSFGDLSLGMTCKPDVNGGSR.Q*	*Oxidation (M)*
											5	*153*	*171*	*2204,98*	*2203,97*	*2203,96*	*0,01*	*0*	*8*	*1,10E+00*	*K.SFTCFTAEVNNNHSAYSQK.E*	
											6	186	207	2347,20	2346,20	2346,20	0,00	0	60	1,50E-05	K.TAAQEPIQPNPASLACELPIAR.R	
											7	226	249	2533,24	2532,23	2532,22	0,00	0	56	4,40E-05	K.APYQIDGSAEASSKPTNPAWLSSR.-	
MYC3	AT4G17880	MYC4	2/4	TAIR8	183	1,60E-14	4	26	10	126	1	515	523	1074,58	1073,57	1073,57	0,00	0	46	7,80E-04	K.IIGWDAMIR.I	
											2	*515*	*523*	*1090,57*	*1089,56*	*1089,56*	*0,00*	*0*	*6*	*9,20E+00*	*K.IIGWDAMIR.I*	*Oxidation (M)*
											3	406	422	1945,98	1944,98	1944,98	0,00	1	38	3,40E-03	R.KPANGREEPLNHVEAER.Q	
											4	177	206	3040,42	3039,41	3039,40	0,01	0	42	5,10E-04	K.GTGLPGQAFSNSDTIWLSGSNALAGSSCER.A	
MYC4	AT4G17880	MYC4 (BAIT)	4/4	TAIR9	483	1,70E-44	2	22	10	436	1	515	523	1074,58	1073,57	1073,57	-0,79	0	59	4,20E-05	K.IIGWDAMIR.I	
											2	515	523	1090,57	1089,56	1089,56	-0,70	0	35	1,10E-02	K.IIGWDAMIR.I	Oxidation (M)
											3	567	578	1472,68	1471,67	1471,68	-3,57	0	71	1,10E-06	K.MGNQFFTQDQLK.V	Oxidation (M)
											4	448	462	1579,85	1578,85	1578,85	-3,18	0	113	7,30E-11	K.ASLLGDAISYISELK.S	
											5	2	17	1830,89	1829,89	1829,89	-2,01	0	91	1,50E-08	M.SPTNVQVTDYHLNQSK.T	
											6	177	206	3040,42	3039,41	3039,40	3,68	0	103	4,60E-10	K.GTGLPGQAFSNSDTIWLSGSNALAGSSCER.A	
MYC4	AT5G20900	JAZ12	4/4	TAIR9	618	5,30E-58	2	53	8	540	1	161	169	1068,50	1067,49	1067,49	-2,29	0	74	6,10E-07	K.NPYPTSDFK.K	
											2	171	181	1130,60	1129,60	1129,60	-1,85	0	62	2,00E-05	K.TDVPTGNVSIK.E	
											3	161	170	1196,59	1195,59	1195,59	-1,42	1	70	2,30E-06	K.NPYPTSDFKK.T	
											4	92	106	1529,79	1528,78	1528,78	-0,06	0	108	4,00E-10	K.NSTSISPVSSPALNR.A	
											5	171	187	1804,90	1803,89	1803,89	-0,14	1	93	8,70E-09	K.TDVPTGNVSIKEEFPTA.-	
											6	10	36	2407,08	2406,07	2406,06	4,60	0	100	8,20E-10	R.ASVEGGCGVADGDGGAAEIGGTGSVEK.S	
											7	107	133	2864,41	2863,41	2863,40	3,15	0	32	7,60E-03	R.APSFSSTSNVASPAAQPFPIQPISFCR.S	

MYC4	AT5G46760	MYC3	4/4	TAIR8	467	6,50E-43	6	23	10	408	1	192	201	1034,56	1033,56	1033,56	0,00	0	56	5,90E-05	R.AGQGQIYGLK.T	
											2	*518*	*526*	*1102,61*	*1101,60*	*1101,60*	*0,00*	*0*	*24*	*1,00E-01*	*K.IIGWDVMIR.V*	
											3	*518*	*526*	*1118,60*	*1117,60*	*1117,60*	*0,00*	*0*	*26*	*7,20E-02*	*K.IIGWDVMIR.V*	*Oxidation (M)*
											4	570	581	1467,67	1466,66	1466,66	0,00	0	27	3,00E-02	K.MGSQFFNHDQLK.V	Oxidation (M)
											5	447	461	1606,87	1605,86	1605,86	0,00	0	84	7,00E-08	K.ASLLGDAISYINELK.S	
											6	*355*	*370*	*1712,79*	*1711,79*	*1711,78*	*0,00*	*0*	*13*	*8,30E-01*	*K.GSNNDEGMLSFSTVVR.S*	
											7	355	370	1728,78	1727,77	1727,78	0,00	0	44	5,20E-04	K.GSNNDEGMLSFSTVVR.S	Oxidation (M)
											8	175	191	1749,86	1748,85	1748,85	0,00	0	107	4,00E-10	R.VIWLSGSGALTGSGCER.A	
											9	316	333	2023,03	2022,02	2022,05	-0,02	1	59	2,40E-05	R.QSSCLVEKDLTFQGGLLK.S	
											10	*545*	*569*	*2782,43*	*2781,42*	*2781,42*	*0,00*	*0*	*6*	*2,90E+00*	*K.ELDLEVNHASLSVVNDLMIQQATVK.M*	*Oxidation (M)*
MYC4	AT4G28910	NINJA	4/4	TAIR8	449	4,10E-41	3	46	14	328	1	276	292	1618,82	1617,81	1617,81	0,00	0	125	6,60E-12	K.DGSGGIVALSQSPFAGR.V	
											2	73	89	1908,87	1907,86	1907,86	-0,01	0	53	5,30E-05	R.SDSGQQPPQNFFNDLSK.A	
											3	179	197	2124,10	2123,10	2123,10	0,00	0	43	7,70E-04	K.EVVRPPTDTNIVDNLTGQR.R	
											4	*349*	*371*	*2295,13*	*2294,12*	*2294,13*	*-0,01*	*0*	*10*	*1,60E+00*	*K.FGGSGARPNLPWVSTTGSGPHGR.T*	
											5	90	110	2345,16	2344,15	2344,15	0,00	1	96	4,20E-09	K.APTTEAEASTKPLWVEDESRK.E	
MYC4	AT1G74950	JAZ2	3/4	TAIR8	291	2,60E-25	2	50	7	224	1	15	24	1211,58	1210,57	1210,58	0,00	0	45	6,20E-04	R.KPSFSQTCTR.L	
											2	128	138	1303,59	1302,58	1302,58	0,00	0	55	2,70E-05	R.VMVFDDFSAEK.A	Oxidation (M)
											3	110	127	1937,01	1936,00	1936,01	0,00	0	86	4,70E-08	K.SVKPESQSAPLTIFYGGR.V	
											4	226	249	2533,23	2532,22	2532,22	0,00	0	39	2,20E-03	K.APYQIDGSAEASSKPTNPAWLSSR.-	
MYC4	AT3G43440	JAZ11	4/4	TAIR8	184	1,30E-14	1	22	3	163	1	80	90	1168,64	1167,64	1167,64	0,00	0	47	3,40E-04	K.NVTGINPALNR.A	
											2	37	55	2039,97	2038,96	2038,96	0,00	0	116	3,90E-11	R.STEPDASTQLTIIFGGSCR.V	
JAZ3	AT3G17860	JAZ3 (BAIT)	2/2	TAIR9	393	1,70E-35	6	59	11	285	1	89	98	1063,55	1062,55	1062,55	0,58	0	90	3,10E-08	K.APYSSVQGVR.M	
											2	88	98	1191,65	1190,64	1190,64	1,56	1	93	1,10E-08	R.KAPYSSVQGVR.M	
											3	42	59	2041,99	2040,98	2040,99	-2,33	0	36	4,10E-03	K.VSASSSQFLSFRPTQEDR.H	
											4	63	87	2727,21	2726,20	2726,20	-1,06	0	41	5,30E-04	K.SGNYHLPHSGSFMPSSVADVYDSTR.K	Oxidation (M)
											5	168	197	3216,60	3215,59	3215,57	7,43	0	23	4,90E-02	R.SSSKPIGSPAQLTIFYAGSVCVYDDISPEK.A	
JAZ3	AT4G28910	NINJA	2/2	TAIR9	510	3,30E-47	4	42	14	396	1	199	212	1509,64	1508,63	1508,63	-1,38	0	88	8,20E-09	R.SNHGGSGTEEFTMR.N	
											2	199	212	1525,63	1524,63	1524,63	-0,59	0	22	2,40E-02	R.SNHGGSGTEEFTMR.N	Oxidation (M)
											3	276	292	1618,82	1617,81	1617,81	0,45	0	141	1,80E-13	K.DGSGGIVALSQSPFAGR.V	
											4	73	89	1908,86	1907,85	1907,87	-7,31	0	63	4,90E-06	R.SDSGQQPPQNFFNDLSK.A	
											5	387	403	2031,88	2030,88	2030,88	-0,28	0	29	7,10E-03	K.IVCACHGSHMSPEEFVR.H	Oxidation (M)
											6	179	197	2124,10	2123,09	2123,10	-2,49	0	62	9,90E-06	K.EVVRPPTDTNIVDNLTGQR.R	
											7	*349*	*371*	*2295,13*	*2294,13*	*2294,13*	*-1,64*	*0*	*12*	*1,10E+00*	*K.FGGSGARPNLPWVSTTGSGPHGR.T*	
JAZ3	AT5G46760	MYC3	1/2	TAIR9	153	1,70E-11	6	18	8	116	1	518	526	1102,61	1101,60	1101,60	-1,05	0	34	1,10E-02	K.IIGWDVMIR.V	
											2	*518*	*526*	*1118,60*	*1117,59*	*1117,60*	*-2,39*	*0*	*24*	*1,10E-01*	*K.IIGWDVMIR.V*	*Oxidation (M)*
											3	447	461	1606,85	1605,85	1605,86	-9,54	0	38	3,60E-03	K.ASLLGDAISYINELK.S	
											4	*355*	*370*	*1728,78*	*1727,77*	*1727,78*	*-3,10*	*0*	*17*	*2,30E-01*	*K.GSNNDEGMLSFSTVVR.S*	*Oxidation (M)*
											5	175	191	1749,87	1748,86	1748,85	3,57	0	27	4,00E-02	R.VIWLSGSGALTGSGCER.A	
JAZ3	AT1G80490/AT1G15750	TPR1/TOPLESS	1/2	TAIR9	/	/	/	/	/	36	1	114	125	1476,79	1475,78	1475,80	-10,80	0	36	6,20E-03	K.EITQLLTLENFR.E	
JAZ5	AT1G17380	JAZ5 (BAIT)	4/4	TAIR8	581	2,60E-54	3	50	10	502	1	33	44	1311,67	1310,66	1310,66	0,00	0	85	6,70E-08	K.GSFGNIDLGLYR.K	
											2	107	117	1322,69	1321,69	1321,69	0,00	0	54	1,00E-04	K.VLVYNEFPVDK.A	
											3	203	215	1491,71	1490,71	1490,71	-0,01	0	89	2,70E-08	R.APYQVNQNAGHHR.Y	
											4	31	44	1568,80	1567,79	1567,80	-0,01	1	39	2,40E-03	K.EKGSFGNIDLGLYR.K	
											5	168	181	1784,84	1783,84	1783,84	-0,01	0	85	5,80E-08	K.EQQQQQEQNQIVER.I	
											6	149	167	2125,01	2124,01	2124,02	-0,01	0	75	4,50E-07	K.SSMVLPDLNEPTDNNHLTK.E	
											7	149	167	2141,01	2140,00	2140,01	-0,01	0	78	2,30E-07	K.SSMVLPDLNEPTDNNHLTK.E	Oxidation (M)
											8	127	148	2494,25	2493,24	2493,25	0,00	0	72	8,60E-07	K.QAKPVTEINIQTPINDENNNNK.S	
JAZ5	AT4G28910	NINJA	4/4	TAIR8	564	1,30E-52	3	44	13	454	1	199	212	1509,64	1508,63	1508,63	0,00	0	82	3,50E-08	R.SNHGGSGTEEFTMR.N	
											2	276	292	1618,82	1617,81	1617,81	0,00	0	129	2,60E-12	K.DGSGGIVALSQSPFAGR.V	
											3	73	89	1908,87	1907,87	1907,86	0,00	0	38	1,80E-03	R.SDSGQQPPQNFFNDLSK.A	
											4	179	197	2124,10	2123,09	2123,10	0,00	0	42	1,10E-03	K.EVVRPPTDTNIVDNLTGQR.R	
											5	349	371	2295,14	2294,13	2294,13	0,00	0	42	9,90E-04	K.FGGSGARPNLPWVSTTGSGPHGR.T	
											6	90	110	2345,15	2344,15	2344,15	-0,01	1	106	3,80E-10	K.APTTEAEASTKPLWVEDESRK.E	
											7	*48*	*72*	*2813,40*	*2812,40*	*2812,39*	*0,01*	*1*	*14*	*4,80E-01*	*K.VIDDFKNFLHPTSQRPAEPSSGSQR.S*	
JAZ5	AT1G15750	TOPLESS	2/4	TAIR8	530	3,30E-49	6	29	23	385	1	150	158	1072,61	1071,61	1071,61	0,00	0	48	3,80E-04	K.LIEANPLFR.D	
											2	159	167	1117,63	1116,63	1116,63	0,00	1	57	4,10E-05	R.DKLQFPTLR.N	
											3	534	543	1212,55	1211,54	1211,54	0,00	0	38	2,20E-03	K.AWLYDNMGSR.V	
											4	103	113	1360,67	1359,66	1359,67	-0,01	0	47	3,90E-04	K.VFSTFNEELFK.E	
											5	114	125	1476,80	1475,79	1475,80	0,00	0	80	2,00E-07	K.EITQLLTLENFR.E	
											6	518	531	1582,81	1581,80	1581,80	0,00	0	65	6,90E-06	K.ENIQFIFSTALDGK.I	
											7	368	388	2227,18	2226,18	2226,20	-0,03	0	50	1,10E-04	K.QTLLLVGTNVGDIGLWEVGSR.E	
JAZ5	AT5G20900	JAZ12	4/4	TAIR8	455	1,00E-41	4	53	7	390	1	161	170	1196,59	1195,58	1195,59	0,00	1	57	4,70E-05	K.NPYPTSDFKK.T	
											2	92	106	1529,78	1528,78	1528,78	-0,01	0	73	1,10E-06	K.NSTSISPVSSPALNR.A	
											3	171	187	1804,89	1803,89	1803,89	0,00	1	68	3,00E-06	K.TDVPTGNVSIKEEFPTA.-	
											4	10	36	2407,09	2406,08	2406,06	0,02	0	123	5,20E-12	R.ASVEGGCGVADGDGGAAEIGGTGSVEK.S	
											5	107	133	2864,41	2863,40	2863,40	0,00	0	70	1,30E-06	R.APSFSSTSNVASPAAQPFPIQPISFCR.S	
JAZ5	AT5G46760	MYC3	2/4	TAIR8	338	5,20E-30	7	24	11	274	1	192	201	1034,56	1033,55	1033,56	0,00	0	61	2,00E-05	R.AGQGQIYGLK.T	
											2	324	333	1091,60	1090,59	1090,60	-0,01	0	59	3,60E-05	K.DLTFQGGLLK.S	
											3	*518*	*526*	*1102,60*	*1101,60*	*1101,60*	*0,00*	*0*	*27*	*6,20E-02*	*K.IIGWDVMIR.V*	
											4	*518*	*526*	*1118,60*	*1117,60*	*1117,60*	*0,00*	*0*	*25*	*8,50E-02*	*K.IIGWDVMIR.V*	*Oxidation (M)*
											5	570	581	1451,67	1450,67	1450,67	0,00	0	39	2,00E-03	K.MGSQFFNHDQLK.V	
											6	*570*	*581*	*1467,67*	*1466,66*	*1466,66*	*0,00*	*0*	*17*	*2,90E-01*	*K.MGSQFFNHDQLK.V*	*Oxidation (M)*
											7	355	370	1712,79	1711,79	1711,78	0,00	0	50	1,40E-04	K.GSNNDEGMLSFSTVVR.S	
											8	*355*	*370*	*1728,79*	*1727,78*	*1727,78*	*0,00*	*0*	*21*	*1,20E-01*	*K.GSNNDEGMLSFSTVVR.S*	*Oxidation (M)*
											9	175	191	1749,86	1748,85	1748,85	0,00	0	38	2,80E-03	R.VIWLSGSGALTGSGCER.A	
JAZ5	AT3G16830	TPR2	1/4	TAIR8	232	2,10E-19	5	14	13	189	1	150	158	1072,61	1071,61	1071,61	0,00	0	60	2,70E-05	K.LIEANPLFR.E	
											2	114	125	1476,80	1475,79	1475,80	-0,01	0	38	3,20E-03	K.EITQLLTLENFR.E	
											3	508	521	1582,81	1581,80	1581,80	0,00	0	62	1,30E-05	K.ENIQFIFSTALDGK.I	
											4	771	790	2018,11	2017,10	2017,10	0,00	0	29	9,80E-03	R.LLYTNSGVGVLALGSNGVQR.L	
JAZ5	AT5G27030	TPR3	1/4	TAIR8	190	3,30E-15	9	11	10	164	1	150	158	1072,61	1071,61	1071,61	0,00	0	60	2,70E-05	K.LIEANPLFR.D	
											2	507	520	1582,81	1581,80	1581,80	0,00	0	62	1,30E-05	K.ENIQFIFSTAIDGK.I	
											3	429	443	1743,92	1742,91	1742,91	0,00	0	42	1,00E-03	K.HLIQLYAFSGPNDLR.Q	
JAZ5	AT1G74950	JAZ2	2/4	TAIR8	98	5,30E-06	10	42	6	52	1	110	127	1937,01	1936,00	1936,01	0,00	0	52	1,20E-04	K.SVKPESQSAPLTIFYGGR.V	

Table S2. List of primers used. Oligonucleotide sequences used within this work to amplify and genotype indicated genes.

Supplemental Table 2

Primer Name	Sequence 5´ to 3´
LBGabiKat8409	ATATTGACCATCATACTCATTGC
LBGabiKat8760	GGGCTACACTGAATTGGTAGCTC
MYC3FwGKgenespecific	AAGGTGGGTTGTTGAAATCTAATG
MYC3RvCDS	TCAATAGTTTTCTCCGACTTTC
MYC4FwCDS	ATGTCTCCGACGAATGTTCA
MYC4RvGKgeneespecific	AACCAAATTCACCACCACCAT
MYC3Fwgateway	**ggggacaagtttgtacaaaaaagcaggcttc**ATGAACGGCACAACATCATCA
MYC3Rvstopgateway	**ggggaccactttgtacaagaaagctgggt**TCAATAGTTTTCTCCGACTTT
MYC3Rvnonstopgateway	**ggggaccactttgtacaagaaagctgggtc**ATAGTTTTCTCCGACTTTT
MYC4Fwgateway	**ggggacaagtttgtacaaaaaagcaggcttc**ATGTCTCCGACGAATGTTCA
MYC4Rvstopgateway	**ggggaccactttgtacaagaaagctgggt**TCATGGACATTCTCCAACTTTC
MYC4Rvnonstopgateway	**ggggaccactttgtacaagaaagctgggtc**TGGACATTCTCCAACTTTC
At5g46830Fwgateway	**ggggacaagtttgtacaaaaaagcaggcttc**ATGATTAATACCGACGATAAC
At5g46830Rvstopgateway	**ggggaccactttgtacaagaaagctgggt**TCAGCTAATTTTCGACATCAA
At5g46830Rvnonstopgateway	**ggggaccactttgtacaagaaagctgggtc**GCTAATTTTCGACATCAA
AtAIBFwgateway	**ggggacaagtttgtacaaaaaagcaggcttc**ATGAATATGAGTGATTTAGGT
AtAIBRvstopgateway	**ggggaccactttgtacaagaaagctgggt**TTATATATCACCAGAGACCTGT
AtAIBRvnonstopgateway	**ggggaccactttgtacaagaaagctgggtc**TATATCACCAGAGACCTGT
MYC3promFw	**cacc**TGTTATTAGCGCAAAGAGGATCG
MYC3promRv	GAAGTTGATTGATGATGTTGTGCCGTTCAT
MYC4promFw	**cacc**ACAGTACTAACGTTTGATGGAAACAT
MYC4promRv	ATCGGTTACTTGAACATTCGTCGGAGACAT
PDF1-2qPCRFw	AGTTGTGCGAGAAGCCAAGT
PDF1-2qPCRRv	GTTGCATGATCCATGTTTGG
VSP2qPCRFw	CGTCGATTCGAAAACCATCT
VSP2qPCRRv	GGCACCGTGTCGAAGTCTAT
JAZ10qPCRFW	GAGAAGCGCAAGGAGAGATTAG
JAZ10qPCRRv	CTTAGTAGGTAACGTAATCTCC
MYC2qPCRFw	GTGCGGGATTAGCTGGTAAA
MYC2qPCRRv	ATGCATCCCAAACACTCCTC
MYC3qPCRFw	TGTTGAAGCAGAGAGGCAGA
MYC3qPCRRv	CTCCGAGAAGCGAAGCTTTA
MYC4qPCRFw	AGGAGCAAACGAGAACTGGA
MYC4qPCRRv	CCATCTCCCCAACCTAACAA
ACTIN-8qPCRFw	CCAGTGGTCGTACAACCGGTA
ACTIN-8qPCRRv	TAGTTCTTTTCGATGGAGGAGCTG
MYC2N-term-attB1-F1old	**ggggacaagtttgtacaaaaaagcaggct**tcATGGAGATTCCGGCACAGGCG
MYC2N-term-attB2-R1	**ggggaccactttgtacaagaaagctgggt**cGGAGGCGCCGGAGAAATCATA
MYC3dom44Fw	**ggggacaagtttgtacaaaaaagcaggct**tcCAGCCTCAGTTCAACGAAGAT
MYC3dom87Rv	**ggggaccactttgtacaagaaagctgggtc**ACTGTGTTATCTCCGGTGGA
MYC2Fw93	**ggggacaagtttgtacaaaaaagcaggct**tcatgTATGATTTCTCCGGCGCCTCC
MYC2Rv160	**ggggaccactttgtacaagaaagctgggt**cCTCCTCATCAACAGCGTCATC
MYC3Fw82	**ggggacaagtttgtacaaaaaagcaggcttc**GACTTCGATTCATCCACCGGA
MYC3Rv141	**ggggaccactttgtacaagaaagctgggtc**TTCATCGTTTGATTCATCGGA
MYC2midFw	**ggggacaagtttgtacaaaaaagcaggctc**GGGATTAGCTGGTAAAGCG
MYC2midRv	**ggggaccactttgtacaagaaagctgggtc**TTAATGAACCGGACTCGGAGT
MYC2NFw	**ggggacaagtttgtacaaaaaagcaggctc**GATGACTGATTACCGGCTACA
MYC2NRv	**ggggaccactttgtacaagaaagctgggtc**TCACGCTTTACCAGCTAATCCCG
MYC2NTFw	**ggggacaagtttgtacaaaaaagcaggcttc**ATGGAGATTCCGGCACAGGCG
MYC2NTRv	**ggggaccactttgtacaagaaagctgggtc**TCACGCTTTACCAGCTAATCCCG
JAZ2FwEcoR1	GTCACGAATTCATGTCGAGTTTTTCTGCCG
JAZ2RvPst-1	GACGTCTGCAGTTACCGTGAACTGAGCCAAG
JAZ3FwEcoR1	CGGAATTCCGGTTCATGGAGAGAGAT
JAZ3RvPst-1	CCAATGCATTGGTTCTGCAGTTTTAGGTTGC
pDEST32Fw	AACCGAAGTGCGCCAAGTGTCTG
pDEST32Rv	CAGAGGTTAGTTCCAACAGCCGA
pDEST22Fw	TATAACGCGTTTGGAATCACT
pDEST22Rv	AGCCGACAACCTTGATTGGAGAC

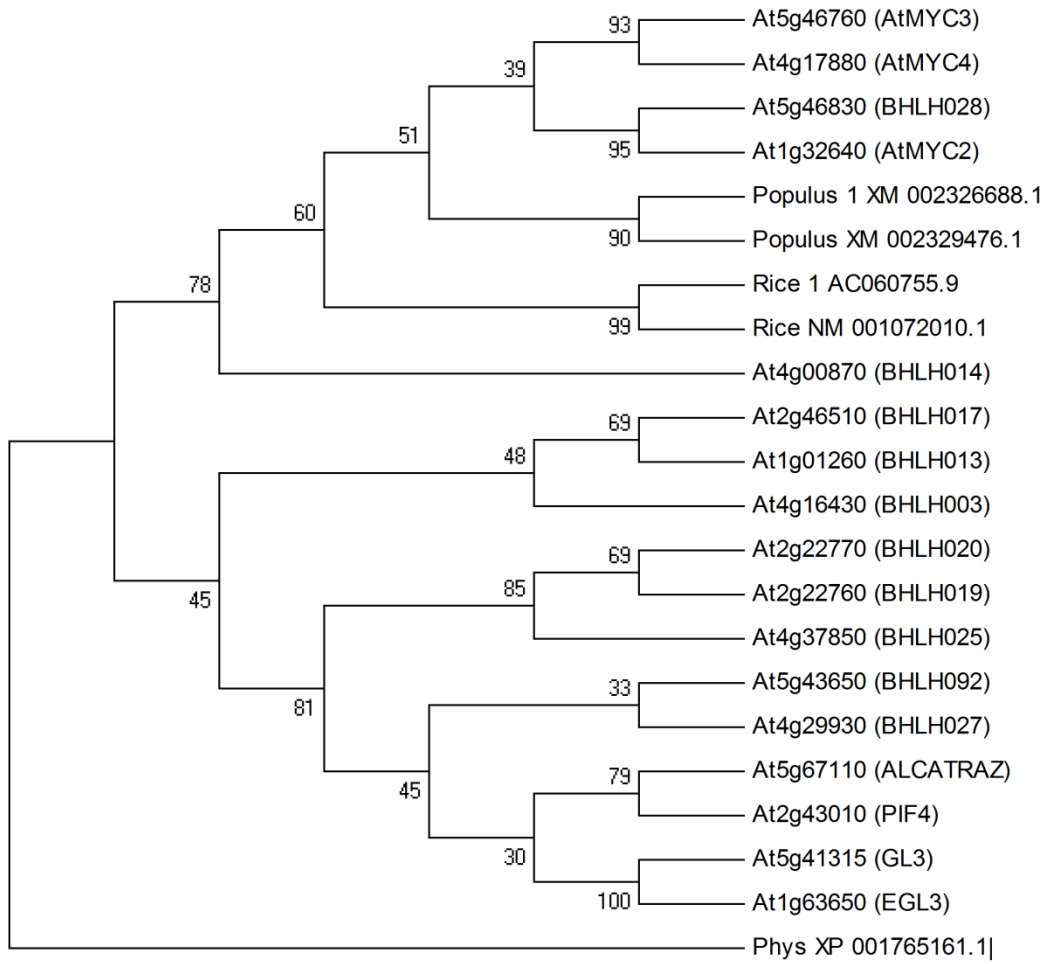


Figure S1. **Phylogenetic tree of MYC2-related proteins from Arabidopsis and other plants.**

Phenogram representation of the Maxima Parsimonia method for 17 Arabidopsis, 2 Populus, 2 Rice and 1 Physcomitrella MYC2 bHLH-related proteins. Sequenced alignment was generated using multiple alignment ClustalW algorithm (T-Coffee web server, (http://tcoffee.vital-it.ch) and the tree was created by MEGA4 program. Bootstrap values based on 100 replicates are shown.

Supplemental Figure 1

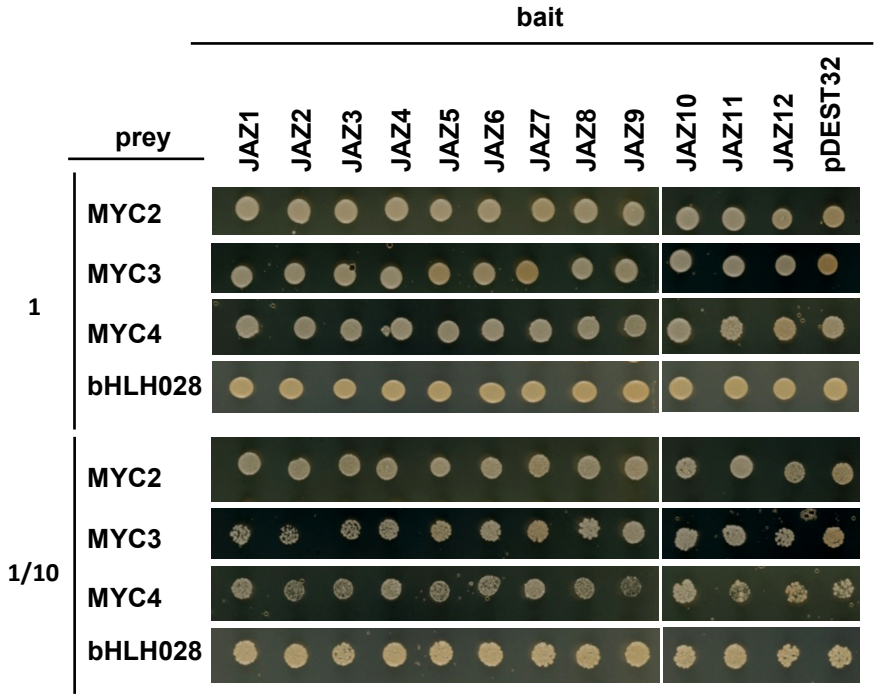


Figure S2. Control growth of yeast cells transformed with MYC3 or MYC4 and with JAZ repressors in yeast two-hybrid assays.
Yeast cells co-transformed with pDEST22-MYC2, pDEST22-MYC3, pDEST22-MYC4 or pDEST22-bHLH028 (preys) and pDEST32-JAZ1-12 (baits) were selected and subsequently grown on yeast synthetic drop-out lacking Leu and Trp (-2), as a transformation control.

Supplemental Figure 2

Supplemental Figure 3

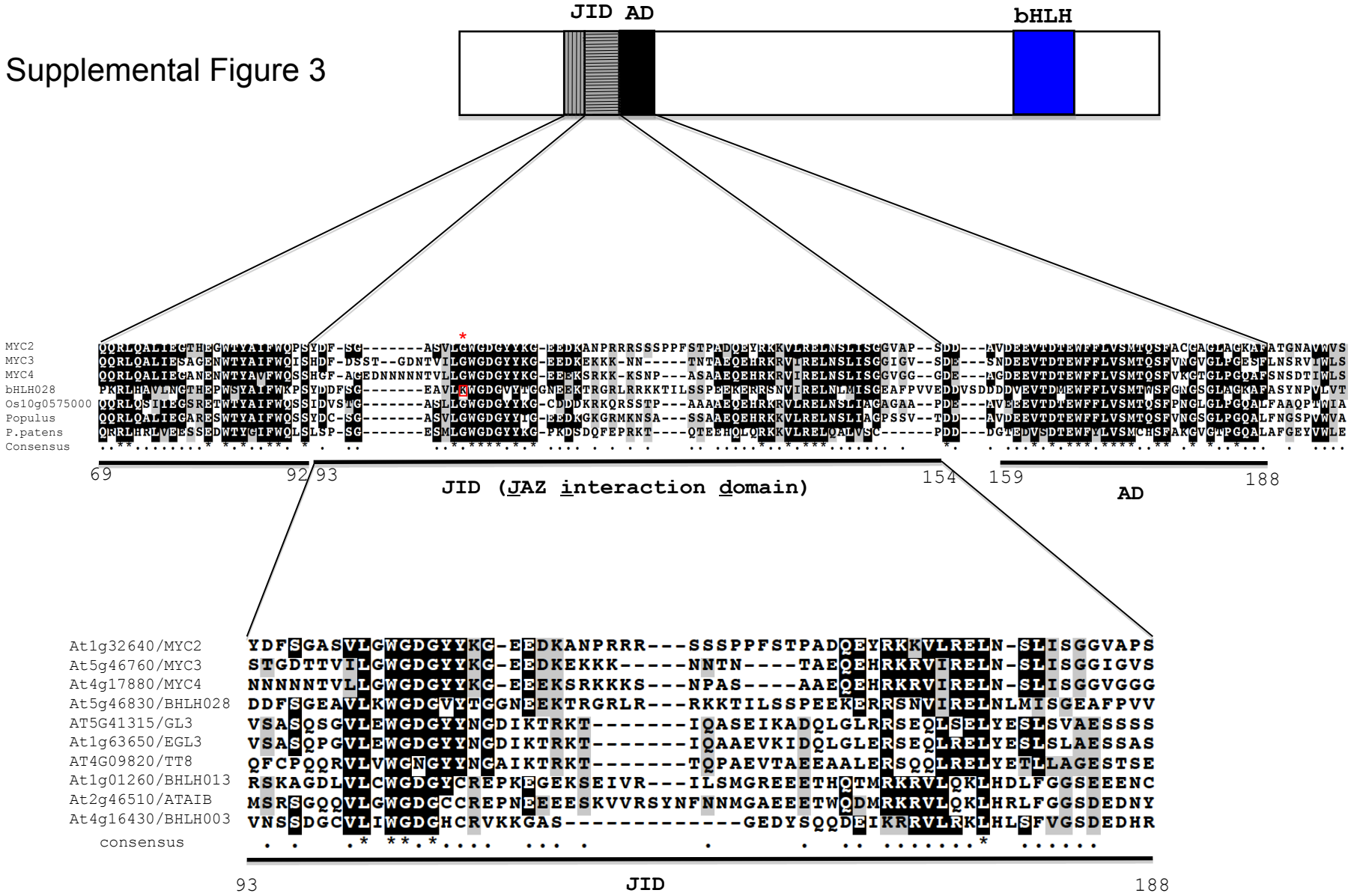


Figure S3. Alignment of the JAZ-interaction domain of MYC2-related proteins.
(A) Aligned sequences of JAZ interacting domain of MYC2, MYC3, MYC4 and At5g46830 from Arabidopsis and MYC2-closest relatives from Rice (AC060755.9), Populus (XM_002329476.1) and Physcomitrella patens (XP_001765161.1). Alignment was created with T-Coffee web server (http://tcoffee.vital-it.ch) using multiple aligment ClustalW algorithm. Residues conserved in all three proteins are labelled by stars, whereas dots indicate residues belonging to the same functional group. The non-conservative change (G-K) in At5g46830 is highlighted with a red square and a red asterisk.
(B) Sequences of the JAZ-interaction domain (JID) of several bHLH proteins of *A. thaliana*: MYC2, MYC3, MYC4,At5g46830 (BHLH028), At5g41315 (GL3), At1g63650 (EGL3), At4g09820 (TT8), At1g01260 (BHLH013), At2g46510 (AtAIB) and At4g16430 (BHLH003). Sequence alignment was generated as in (A).

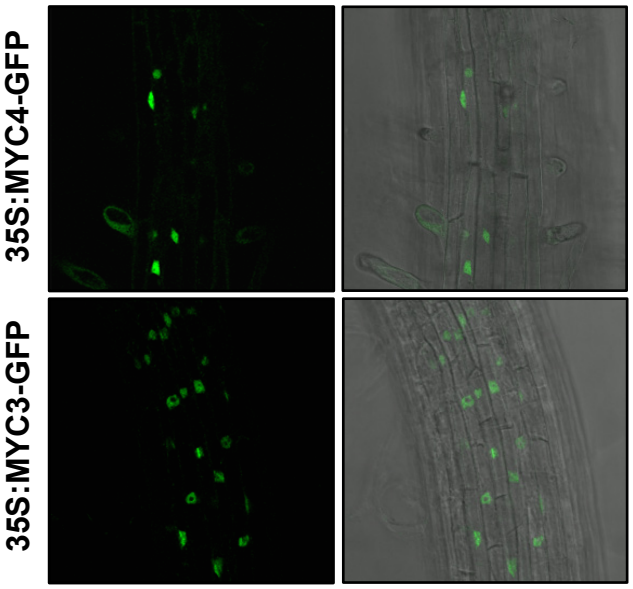


Figure S4. MYC3 and MYC4 are nulear-localized bHLH.
Confocal microscope visualization of nuclear-localized MYC4-GFP and MYC3-GFP fusion proteins in the roots of 35S:MYC4-GFP (upper panels) and 35S:MYC3-GFP (lower pannels) transgenic Arabidopsis seedlings.

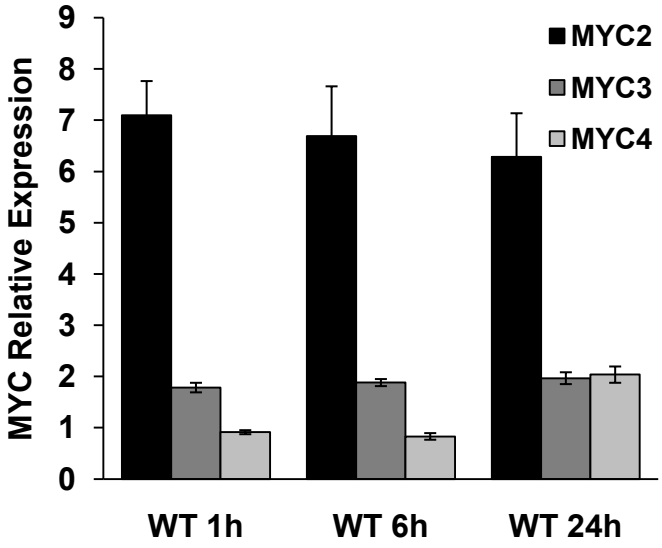
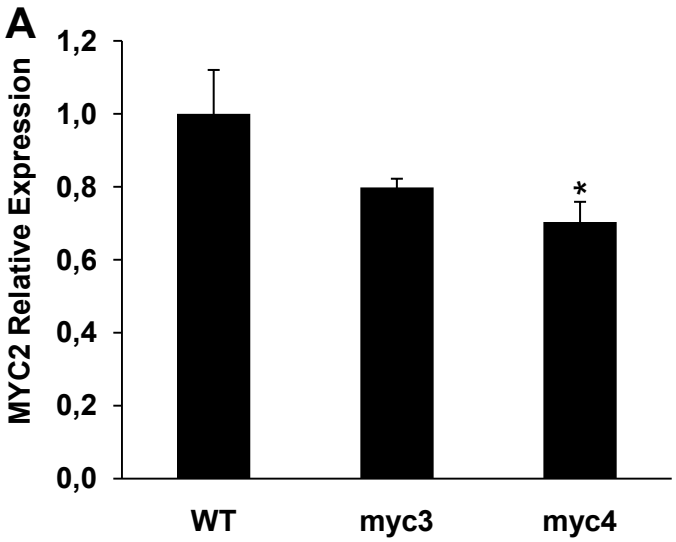
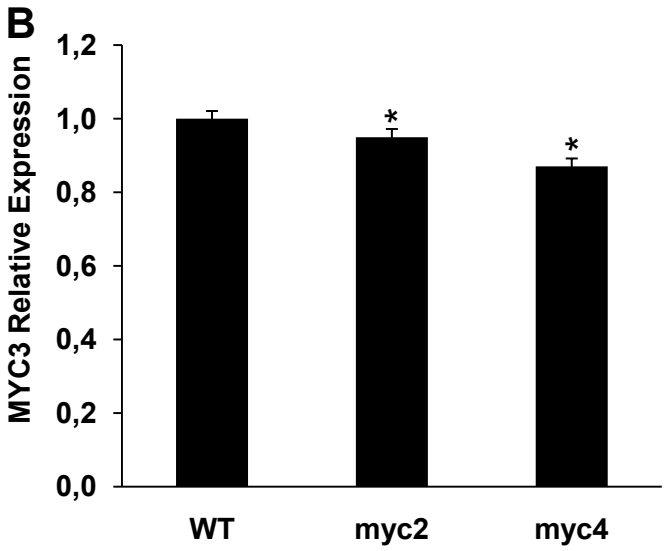


Figure S5. **JA dependent induction of *MYC3* and *MYC4* gene expression.**
Quantitative RT-PCR analysis of *MYC2, MYC3* and *MYC4* JA dependent induction. 8-days-old WT (Col0) seedlings were assayed for the expression of these three genes in a time-course experiment (1, 6 and 24 hours) in response to 50 μM JA. The measurements (three technical replicates) represent the ratio between mock and treated (50 μM JA) plants and the level of each gene is relative to ACTIN8.





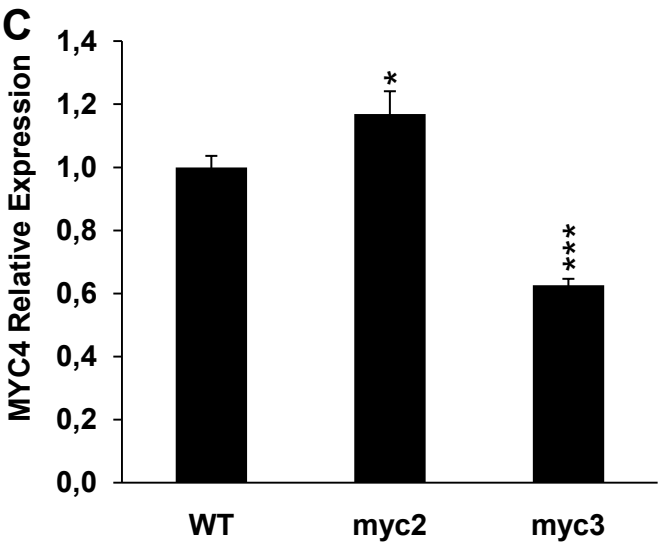


Figure S6. Expression of MYC genes in *myc* mutant backgrounds.
MYC2, MYC3 and *MYC4* expression was analyzed in WT (Col-0) and mutant backgrounds *myc2, myc3* and *myc4.* Quantitative RT-PCR measurements were carried out in 8-days-old seedlings growing in control media. Measurements (three technical replicates) represent the level of each gene relative to ACTIN8 normalized to WT (Col-0) values.

Supplemental Figure 6

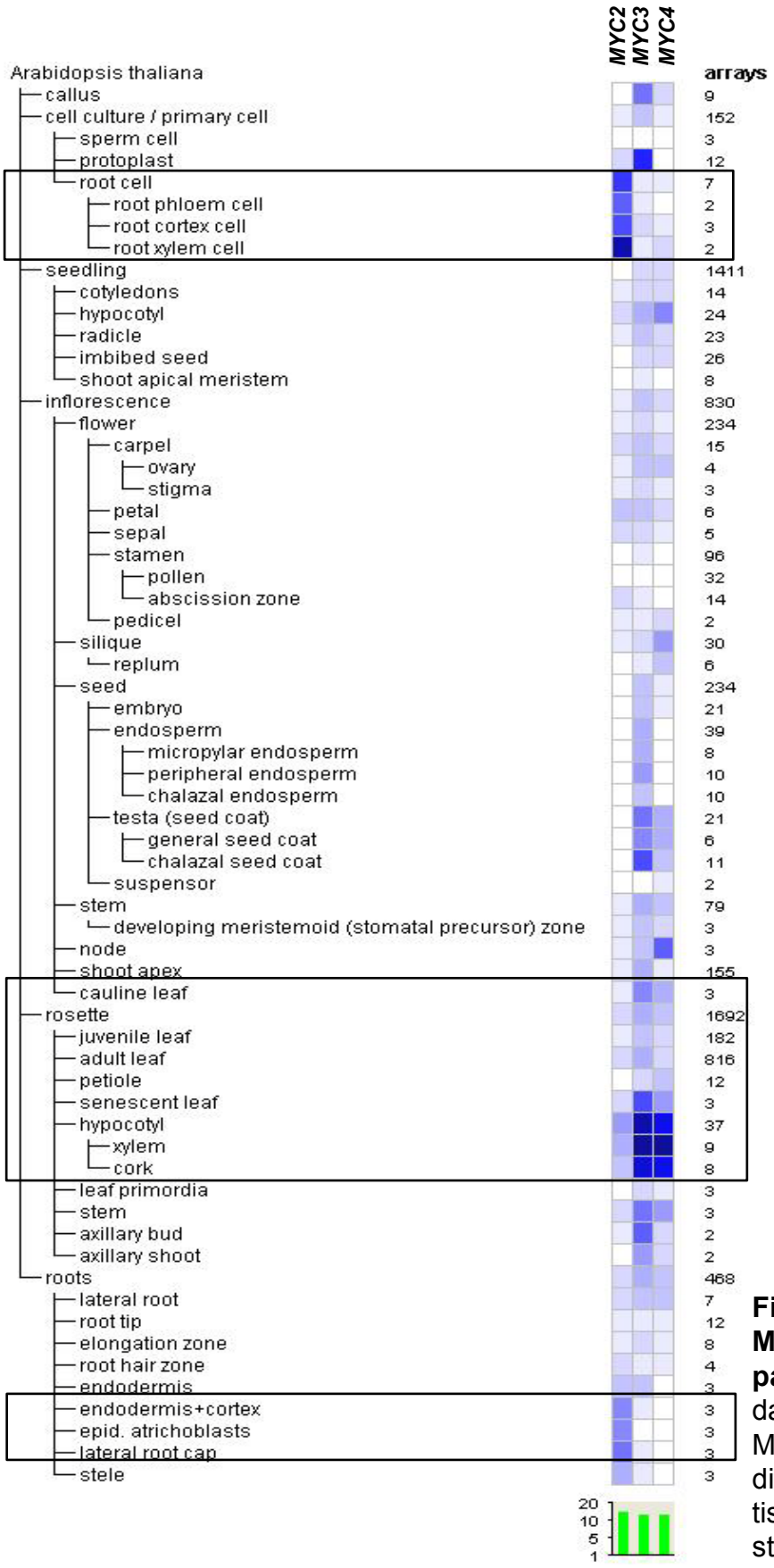


Figure S7. MYC2, MYC3 and MYC4 tissue expression patterns. Genevestigator array data of MYC2, MYC3 and MYC4 expression levels in different *Arabidopsis thaliana* tissues and developmental stages.

Supplemental Figure 7