**Results :**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Strains** | **Gene** | **Chromosome** | **Position** | **Mutation type** |
| Gar12 | *GAI* | I | 5'149’424 | 3bp inframe deletion (ATC) |
| *GAI* | I | 5'149’496 | 52bp frameshift Deletion |
| *SPY* | III | 3'637’438 | Missense mutation: T>C |
| Gar13 | *GAI* | I | 5'149’424 | 3bp inframe Deletion (ATC) |
| *GAI* | I | 5'149’495 | 51bp inframe Deletion (DELLA) |
| *GAI* | I | 5'149’624 | 1bp frameshift deletion (G) |
| *SPY* | III | 3'637’438 | Missense mutation: T>C |

**Table xx**: Table showing all non-synonymous mutations found in *Arabidopsis Thaliana* strains Gar12 and Gar13.

We screened two mutant strains of *Arabidopsis Thaliana* that were generated by random γ-ray mutagenesis of CS63 for mutations in gene *GAI* and *SPY*. C63 contains a 51bp deletion in the DELLA-domain of *GAI*. The deletion acts as a gain-of-function mutation in DELLA, thus resulting in a dwarf phenotype due to reduced GA-signalling. As opposed to C63, Gar12 and Gar13 exhibit normal growth and we hypothesized that random mutation in *GAI* and/or *SPY* may be causative for their phenotypic reversion.

We found that Gar13 had the original mutation in the DELLA-domain of the *GAI* gene, whereas Gar12 had a deletion at the same location, but affected 52 nucleotides instead of 51 like in the original deletion found in C63 (Table xx). Furthermore, we found one additional frameshift deletion in the functional GRAS-domain of strain Gar13 (Table xx). The only mutation in *SPY* was a mutation affecting only one single amino acid that was present in both strains.

**Discussion:**

Due to the additional nucleotide affected by the large deletion in Gar12, the deletion leads to a frameshift that affects all codons upstream of the DELLA-domain and thus substantially affects the structural integrity of DELLA. This means that although the *GAI* gene exhibits a gain-of-function mutation, which renders the protein resistant to proteasomal degradation, the deletion changes a substantial number of amino acids in the functional GRAS-domain. Therefore the repressive effect of DELLA is subverted and the plant is able to thrive (Figure 1A).

In the Gar13 strain, the gain-of-function deletion of 51bp of the DELLA region is present, meaning that the phenotypic reversion is not given by the presence of the DELLA domain, but rather by another mutation in the functional domain. We assume that the only mutation that could be able to abrogate the gain-of-function mutation DELLA is a single nucleotide, which induces a frameshift in the reading frame and thus probably affects the functional GRAS-domain (Table xx and Figure 1). Similar to Gar12, this strain is able to grow normally, due to inactivation of the DELLA protein (Figure 1).

Ein Bild, das Screenshot enthält.

Automatisch generierte Beschreibung

**Figure 1:** Illustrative summary of the project. (A) The DELLA protein is shown with its main domains. Regions highlighted in red represent deletions that were found in Gar12 and/or Gar13.

The other 3bp deletion, which was discovered downstream of the primary deletion, is probably redundant as the 51bp deletion prevents DELLA from binding to GA-loaded GID1 regardless of other mutations. Furthermore, this variant is found in both Gar12 and Gar13, which suggests that the mutation did not arise from random mutagenesis, but was probably already present in C63.

Since loss-of-function mutations in *SPY* are known to suppress gain-of-function mutations in *GAI*, we also searched for potential non-synonymous mutations in *SPY*. However, in both strains, we only found one missense variant in the *SPY* gene, changing a valine to alanine in the amino acid chain. This mutation is unlikely to have an impact on the function of SPY since the amino acid substitution (V to A) is a conservative replacement with a low impact on biochemical properties. Moreover, similar to the 3bp inframe deletion in *GAI*, this mutation was also found in both Gar12 and Gar13 and was therefore presumably present in the original plant C63. Taken together, Gar12 and Gar13 do not exhibit the dwarf phenotype due to mutations in the functional GRAS-domain of *GAI* rather than loss-of-function mutations in *SPY*  (Figure 1B).