| Mutant (total) | Actual | | | Expect for release of TGS | | | P-value | Expect for no release of TGS | | | P-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | GFP+ | GFP- | GFP+ (%) | GFP+ | GFP- | GFP+ (%) | | GFP+ | GFP- | GFP+ (%) | |
| *rdr2* (308) | 23 | 285 | 7.5 | 101.1 | 206.9 | 32.8 | $2.6x10^{-21}$ | 57.8 | 250.2 | 18.8 | $3.9x10^{-7}$ |
| *rdr6* (308) | 36 | 272 | 11.7 | 101.1 | 206.9 | 32.8 | $2.8x10^{-15}$ | 57.8 | 250.2 | 18.8 | $1.4x10^{-3}$ |
| *nrpd1* (262) | 37 | 225 | 14.1 | 86.0 | 176.0 | 32.8 | $1.1x10^{-10}$ | 49.1 | 212.9 | 18.8 | $5.5x10^{-2}$ |
| *ago4* (616) | 115 | 501 | 18.7 | 202.1 | 413.9 | 32.8 | $7.8x10^{-14}$ | 115.5 | 500.5 | 18.8 | $9.6x10^{-1}$ |

**Supplementary Table 1:** Effects of mutations on *GFP* silencing.

To determine whether *GFP* silencing is released in *rdr2*, *rdr6*, *nrpd1* and *ago4* mutants, F2 seeds (segregating for the target locus, silencer locus and mutation) were sown on sterile medium and the number of seedlings showing GFP fluorescence in both the root and shoot apical meristems was assessed approximately 20 days after germination. If a mutation releases silencing, approximately one-third of the F2 progeny should be GFP-positive; if a mutation does not release silencing, only around 18.5% of the F2 progeny should be GFP-positive. The results (actual percentages of GFP-positive shown in red) indicate that the *rdr2-1*, *rdr6-1* and *nrpd1-7* mutations do not release *GFP* silencing. These findings were confirmed by genotyping, which demonstrated the existence of GFP-negative plants that contained the target and silencer loci and were homozygous for a given mutation.

For *ago4-1* the picture was less clear; 115/616 (18.6%) F2 seedlings were positive as assessed by GFP fluorescence in both shoot and root apical meristems, but a number of additional seedlings showed fluorescence in root meristem only. To adhere to a uniform criterion for release of silencing, these were not counted as revertants in the present study. However, of the 44 F2 plants that contained the target locus and silencer locus (determined by genotyping) and were GFP+, all were homozygous for the *ago4-1* mutation. In addition to partial redundancy of AGO4 and AGO6 on targets of RNA-directed DNA methylation (Zheng et al., 2007), the *ago4-1* mutation is in the Landsberg erecta background and this may affect the uniformity of GFP reactivation. P-values were calculated by a chi-test.

Zheng X, Zhu J, Kapoor A, Zhu, J-K (2007) Role of *Arabidopsis* AGO6 in siRNA accumulation, DNA methylation and transcriptional gene silencing. *EMBO J* **26**: 1691-1701