Combination of mutations *sil* and *ins*2 can cause conversion of stipules into compound leaves

Berdnikov, V.A. and Gorel, F.L.
Inst. of Cytology and Genetics
Russian Acad. of Sci., Novosibirsk, Russia

Previously we described the *ins*2 mutation, which caused angular incisions in the leaflet tips (1). Within such incisions, the central veins of the leaflets were transformed into rachillae bearing unbranched tendrils. This phenotype becomes more pronounced from node to node, up the plant. At flowering nodes, leaflets may lose their laminae and be converted into compound pinnae typical for *af* plants. In the *ins2/ins2 tl/tl* double homozygote, the structure formed at the base of leaflet incision may look like the pinnate leaf of a *tl* mutant, specifically a rachis bearing normal oval leaflets.

It was shown by Marx (3) that, in plants homozygous for the mutation *sinuate leaves (sil)*, the distal portions of stipules acquire some properties of leaflets. In plants homozygous for *sil*, the chimeric nature of stipules can be easily detected in the background of mutations differentially affecting the wax coat of stipules and leaflets. For example, the *wlo (supra-incerata)* mutation, removes wax from the upper surface of leaflets and also from the leaflet-like distal portion of the *sil* stipules (3). Most impressively the *sil* effects are manifested in the *af/af* background (3): the stipules of *sil/sil af/af* plants are often split with adventitious structures looking like an *af* leaf arising from the base of the cleft. Recently, Husbands et al. (2) described effects of *sil* on the stipule phenotypes in *Af/Af* and *af/Af* backgrounds. They showed that under short day conditions the stipules of *sil/sil, af/Af* plants were converted into compound leaves with

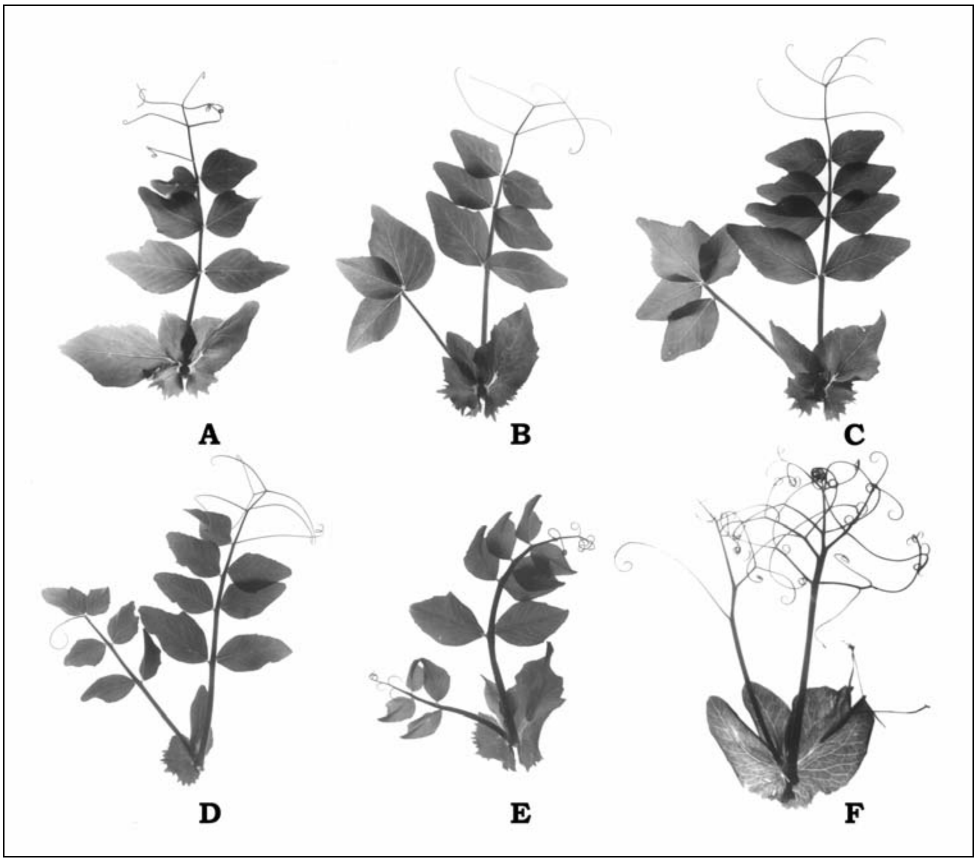

Fig 1. Leaves at successive nodes of sil wlo ins2 Tl *plants. A, B, C, D and E - nodes 23, 25, 26, 27, and 28, respectively. In D one stipule is removed. F, leaf of the double* sil af *mutant (line W15311), node 12.*

their own stipules. In present paper we describe a similar transformation of the stipules in plants homozygous for *sil* and *ins*2.

In the double *sil af* mutants (lines W15110 and W15311 from the Marx collection at the USDA Western Regional Plant Introduction Station, Pullman, Washington), we observed that the leaflet-like segment of the stipule is split and its main vein is transformed into a structure indistinguishable from the distal part of a compound pinna typical for the *af* leaf (Fig. 1F). At the same time, in contrast to observation of Husbands et al (2), in *sil wlo Af* plants (line W15460 from the Marx collection) we never found cleaved stipules, although the stipules always had the waxless diamond-shaped regions at their tips. Subsequently, we will refer to these regions covered with cuticle typical for leaflets, as leaflet-like regions (LLR).

Plants were grown in the greenhouse in hydroponic claydite beds fed by standard Knop nutrient solution under long day conditions. They were illuminated by 8 hr daylight/ 16 hr incandescent light of 7,000 - 10,000 lx intensity. One plant of the line W15460 (*sil wlo tl Ins*2) was crossed with our line AFD (*Sil Wlo Tl ins*2), and among 152 F_2 plants six individuals with incisions on leaflets and waxless LLRs on the stipules were selected. All of them had tendrils with narrow laminae characteristic for the heterozygotes *tl*/*Tl*. Among their progenies from selfing several exceptional individuals with a strikingly altered stipule morphology were observed, although their leaflets were practically devoid of incisions. Two such plants (one homozygous for *tl* and one homozygous for *Tl*) were selected for further analysis. All the offspring from the cross of exceptional plants with the lines bearing wild-type *Ins*2 alleles had cuts in the leaflets that indicated that the *sil* parents were homozygous for *ins*2.

Fig. 1 shows the changes in stipule morphology in the triple homozygote *ins*2/*ins*2 *sil*/*sil wlo*/*wlo*. At higher nodes (>15) the stipule is often transformed into a tripartite structure, two lateral stipule-like parts and the central one developed from the waxless LLR. The central part tends to be more complex at successive nodes. Several stages of complexity can be distinguished. First, the waxless LLR separates as a lobe which looks like a large leaflet with a short petiolule resembling the leaf of the *uni* mutant (Fig. 1A); 2) LLR is converted into a compound leaf without tendrils (Fig. 1B,C); 3) LLR becomes similar to the leaf of uni^{tac} mutant with the terminal leaflet and subterminal tendrils (Fig. 1D); and 4) LLR is transformed into an adventitious compound wild-type leaf with the terminal tendril (Fig. 1E). Thus, we can see that the extreme form of stipule transformation looks like the wild-type leaf with both stipules. The maximum expression of aberrant phenotype is observed at pre-flowering nodes. It should be noted, that the leaves subtending flowers have normal stipules.

In the *sil ins*2 *tl wlo* mutant, the adventitious leaf developed from LLR looked like a unipinnate leaf of the *tl* mutant (Fig. 2). It is of interest to note that the leaflets as a rule lacked incisions in spite of the presence of *ins*2. The first flowers appear very late (at nodes > 30), and the leaves subtending flowers are normal. Often the plants do not flower at all.

Earlier we showed that *Ins*2 is a synergist of *Af*, and its effect becomes stronger in the *af*/*Af* background (1). Stipule phenotypes of the *sil ins*2 plants (Fig. 1) display a striking resemblance to those of the *sil*/*sil af*/*Af* plants presented by Husbands et al (2). It seems that the homozygote for *ins*2 can mimic the action of the heterozygote *af*/*Af*.

Thus, using combination of *sil* and *ins*2 mutations, we showed that the original cluster of

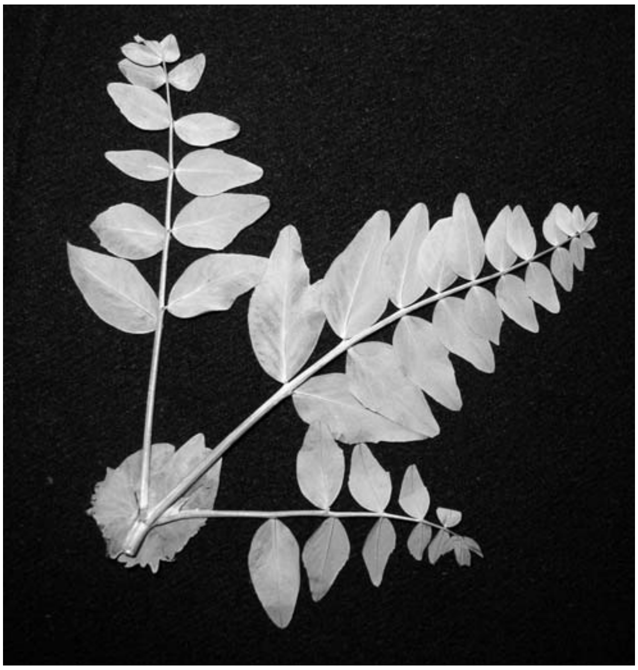

Fig 2. Leaf at the node 32 of the sil wlo ins2 tl *plant.*

three stem appendages (one rachis and two stipules) tend to transform into seven appendages, three rachises and four stipules. It is important to notice that the stipules between rachises have a leaflet-like sector and in principle may generate new rachises and stipules between them. Thus, in *sil* mutants, there is a morphogenetic potency to initiate repetitively primordia of rachises and stipules around the shoot apex. The continuation of this morphogenetic activity may eventually result in formation of a whorl of alternating leaves and stipules. The generation of such whorls during ontogeny of *Acacia longipedunculata* was described by Rutishauser and Sattler (4). Thus, orthologs of *Sil* may have some relation to genetic mechanisms of nodal whorl formation in evolution.

Acknowledgment: This work was supported by the Russian State Program 'Russian Fund for Fundamental Research', grant No 02-04-49426.

1. Berdnikov, V.A., Gorel', F.L., Bogdanova, V.S. and Kosterin, O.E. 2000. Pisum Genetics 32: 9-12.
2. Husbands, A., Emirzade, T. and DeMason, D. 2003. Pisum Genetics 35: 6-9.
3. Marx, G.A. 1977. Amer. J. Bot. 64: 273-277.
4. Rutishauser, R. and Sattler, R. 1986. Can. J. Bot. 64: 1987-2019.