Multiple Regulatory Roles for *SELF-PRUNING* in the Shoot System of Tomato

In the *Scientific Correspondence* by Thouet et al. (2008), the authors present several sections of tomato (*Solanum lycopersicum*) apices probed by the *SELF-PRUNING* (*SP*) gene and claim that (1) *SP* is expressed in all nongrowing axillary meristems, not only sympodial meristems, and (2) *SP* is not expressed at all in all major organ primordia, contrary to what we published (Pnueli et al., 1998). Based solely on this evidence and a selected subset of the available literature, they call for a revision of *SP*, as a pleiotropic gene promoting growth and thus, among other functions, the cycling of flowering in the sympodium (Pnueli et al., 1998, 2001). Instead, they restrict the function of *SP*, well as all *TERMINAL FLOWER1* (*TFL1*) orthologs, to nongrowing axillary meristems, perhaps for regulation of branching.

The in situ results are indeed different from ours, but in situs of rare transcripts are problematic because slight modifications in the procedures or in plant material may result in inflated differences. We note that the cultivar we used was different from theirs, as divergent are, for example, the Columbia and Landsberg *erecta* Arabidopsis (*Arabidopsis thaliana*) ecotypes. These technical limitations are indeed evident from the order of magnitude differences in their own results (compare their figure 1E with 1B and F or 3A). We agree, however, that *SP* is expressed in axillary meristems: In Pnueli et al. (1998), we stated, ''It is of interest that the *SP* gene is expressed at a particularly high level in all axillary buds along the shoot even though only some of these buds ultimately give rise to side branches'' (p. 1986).

However, in contrast to Thouet et al. (2008), *TFL1* orthologs are expressed, as specified below, in many organs in diverse plant species, although, as expected of a regulatory pleiotropic gene, not in all of these organs in every plant species. Also in contrast to what Thouet et al. (2008) said, *SP*, and other *TFL1/CENTRORADIALIS* (*CEN*) homologs function, as cited below, outside of nongrowing axillary meristems.

SP transcripts were detected by PCR in RNA from leaves, flowers, and stems of tomato (Carmel-Goren et al., 2003). *TFL1* RNA is found in vegetative and reproductive organs of Arabidopsis (AtGenExpress and see also Sohn et al. [2007]), in leaves and flowers of pea (*Pisum sativum*; Foucher et al., 2003), and in all organs of rice (*Oryza sativa*). In rice, *TFL1* genes were detected also by in situ in stems, leaf primordia, and vascular strands (Zhang et al. 2005). Thouet et al. (2008) cite evidence that ryegrass (*Lolium perenne*) *TFL1* ''also'' is expressed exclusively in nongrowing meristems (figure 3 in Jensen et al., 2001). But this evidence, although not noted by Thouet et al., was actually obtained by expressing the *pLpTFL1:GUS* gene in Arabidopsis, not in ryegrass. In ryegrass, *LpTFL1* is expressed in leaves, and thus Jensen et al. (2001) commented, ''No GUS expression was detected in the apical meristem or in Arabidopsis leaves, although *LpTFL1* is expressed in ryegrass leaves'' (p. 1523). Particularly relevant is also the expression of *TFL1* genes in stems and all floral organs in citrus (Pillitteri et al., 2004). Finally, an excellent evidence for the expression of SP in leaves was provided by the Louvain-La-Neuve lab (Quinet et al., 2006), who chose to isolate *SP* cDNA clones from, of all other possible sources, tomato leaf RNA.

Thouet et al. (2008) note, ''Such a widespread pattern was unexpected because the *sp* mutation has no pleiotropic effects on the architecture of the initial segment, leaves, or inflorescences. This curious situation has been stressed by several authors…'' (p. 61). However, in every pair of isogenic lines, internodes of *sp* plants are shorter and this was known and stated in Pnueli et al. (1998). A possible function for a *CENL* gene in stems elongation was also proposed in *Populus* (Ruanala et al., 2008). *SP* interacts with *JOINTLESS1* to regulate leafiness in the inflorescence (Rick and Butler, 1956; Szymkowiak and Irish, 2006), and a mutation in the *SP* gene increases ramification of the inflorescence and fasciation of the flower in response to hormonal treatment (Cordner and Hedger, 1959). And the function of *TFL1* is critical for the evolution of inflorescence architecture (Prusinkiewicz et al., 2007). And in contrast to what they say, *uf sp* plants flower earlier and more extensively than *uf* alone, as shown by Quinet et al. (2006), table 4. More recently Wang et al. (2008) reported that PPF1 may suppress plant senescence (a character of leaves) via activating *TFL1* in transgenic Arabidopsis plants.

Axillary and sympodial branching represent two discrete regulatory programs of branching. All types of branching have common elements, but sympodial branching, per definition, depends on the prior termination by a determinate inflorescence in tomato or by seasonal growth cessation in woody sympodial systems. In many backgrounds in tomato, such as in *lateral suppressor* (Malayer and Guard, 1964) or in *single flower truss* (*sft*) plants (Lifschitz and Eshed, 2006), sympodial branching is completely uncoupled from axillary branching. The reference cited by Thouet et al. (2008) to an increased axillary branching in *sp* plants (Kinet and Peet, 1997) is hard to judge if the numbers, critical distribution of branches, and interactions with known branching genes are missing. In pea, an up-regulated

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TFL1 ortholog delays flowering and rather induces excessive branching. Overexpression of *SP* in decapitated tomato or of *TFL1* in Arabidopsis (Ratcliffe et al., 1998) does not arrest branching as would be perhaps expected according the revised view of *SP* function.

Finally, the identification of *SFT*, a functional antagonist of *SP*, with florigen (Lifschitz et al., 2006), and the possibility that *TFL1* is also mobile (Conti and Bradley, 2007) make discussions of *SP* more meaningful in the context of florigen. This we attempted in our review (Lifschitz and Eshed, 2006), and a detailed discussion is being prepared for publication.

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Response to Lifschitz Letter

Professor Lifschitz's *Letter to the Editor* is constructed on his assertion that, in our recent article (Thouet et al., 2008), we ''restrict the function of *SP*, well as all *TERMINAL FLOWER1* (*TFL1*) orthologs, to nongrowing axillary meristems,'' which is not the case. We want to clarify this main point and the answers to other concerns will emerge.

The *SELF-PRUNING* (*SP*) gene is known to control sympodial growth in tomato (*Solanum lycopersicum*), since mutants show successive sympodial units of reduced size and, eventually, determinate growth. Sympodial growth starts when the shoot apical meristem (SAM) initiates the first inflorescence; the uppermost axillary meristem (the sympodial) then takes over. We therefore analyzed the expression pattern of *SP* around the time of floral transition of the SAM and performed in situ hybridizations in shoot apices, which comprise the SAM, leaf primordia, and their axillary meristems. Since the *SP* gene is orthologous to *TFL1*, we were surprised to observe that *SP* was not expressed in the vegetative or inflorescence SAM, but was restricted to axillary meristems, including the sympodial. Transcript level was high until these meristems started growing.

In any scientific report, the experimental approach defines the context of the discussion and the limits of its conclusions. We analyzed shoot apices, which,

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