Origin of petiole and midrib in *Arabidopsis thaliana*

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Reporter genes encoding β-glucuronidase (*GUS*), green fluorescence protein and luciferase are used for studying plant development. These are tagged with transposons or tissue-specific promoters and their expression pattern reflects the plant developmental stages. However, in case of leaf development, there is a lack of easily identifiable markers of cell states that precede procambium formation. In our work of analyzing spontaneous mutation frequencies in *Arabidopsis* using mutated *GUS* gene, we obtained a plant which reveals the origin of petiole and midrib in *Arabidopsis* leaf. The frequency of spontaneous homologous recombination was very low (10^{-6} events per cell division) and we observed that recombination in the truncated *GUS* gene had taken place in one of the cells of the leaf primordium and, both petiole and midrib generated from this particular cell. This is the first report revealing the most probable origin of petiole and midrib from leaf primordium.

Leaves are one of the most specialized organs of the plant shoot system. Scales, bracts and floral organs including petal, sepal, stamen and carpel are modified leaves [7, 12]. Also, leaves are more relevant organs for photosynthesis and respiration.

Leaves arise at predictable positions around the periphery of the shoot apical meristem. They are produced by several external layers of cells called primordia. A leaf primordium usually originates from at least three cell layers of the shoot meristem [17]. The size of a leaf primordium varies in different species, ranging from approximately five to 10 cells per layer in *Arabidopsis* [4, 5] and upto 100 cells per layer in tobacco [14], and cotton [2]. Typically, leaves comprise of a leaf base, a basal stalk (known as a petiole or rachis) and a green and flat portion called the blade or lamina [12]. Though morphological features, such as the dorsiventral polarity of the leaf primordium and the subdivision of the lamina into lobes or pinnae, are decided very early in leaf development, histological aspects of leaf identity are determined only after a leaf primordium is already well established [12, 19]. Due to the difficulties encountered in studies of the complex leaf development that involves simultaneous division and elongation of cells, the details of leaf development, in particular in dicotyledonous plants such as *Arabidopsis*, remain unclear [4, 13].

The reporter gene encoding β-glucuronidase (*GUS*), inactivated by the insertion of the maize *Activator* (*Ac*) transposon was previously deployed to trace the developmental pattern in plants because *Ac* transposition during early plant development would result in sectors of clonally related GUS-positive cells in these plants [10]. For example, the trichome spacing pattern in *Arabidopsis* leaves was studied by following the GUS positive sectors in them [9]. Donnelly *et al*. (1999) analyzed the expression pattern of *cyc1At::GUS*, which is the fusion of *GUS*gene with a cyclin gene (*cyc1At*, a specific marker of the G2/M phase of the cell cycle) and studied the division and enlargement of leaf cells in

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leaf primordia from the first protrusion to leaf maturity. A common spatiotemporal pattern of early vein formation was revealed by the characterization of expression profiles of *GUS* gene fused to four markers (*Athb8-GUS*, *553-643*, *ET1335* and *GT5211*) that expressed in continuous domains connected to pre-existing vasculature [16]. The recent advances in leaf development are based on studies on mutants involving alteration in auxin levels, microRNAs (miRNAs) regulation and expression of regulatory transcription factors [15].

In our lab, we work on analysis of spontaneous somatic mutation frequencies using transgenic *Arabidopsis thaliana* plants with mutated *GUS* gene for detecting point and frame shift mutations, and homologous recombinations (HR). For this, respective alterations were made in the CaMV 35S promoter-driven reporter gene *GUS* [8, 1, 11]. In case of reversion of point or frame shift mutations in mutated *GUS*, or occurrence of a double homologous

recombination in the truncated invert/direct *GUS* construct (Fig. 1A), the functional *GUS* gene encoding β-glucuronidase enzyme will be transcribed. Cells possessing the *GUS* product alone will stain blue (Fig. 1B) when subjected to the substrate 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid (Jefferson 1989). The blue spots are randomly located in the plants and each blue spot in the plant is derivative tissue from a single cell [8, 11].

Upon analyzing 19000, 11000 and 900 plants with mutated *GUS* to detect point mutations, HR and frame shift mutations, respectively, we identified a single plant where the petiole and whole midrib of a leaf stained blue after performing GUS histochemical staining (Fig. 2). This plant carries two segments of the *GUS* reporter gene, sharing 566 bp of sequence homology arranged in inverted orientation, and only a HR event can make the gene functional (Fig. 1A) [11, 18], thereby giving a blue spot upon histochemical staining (Fig. 1B). The blue-
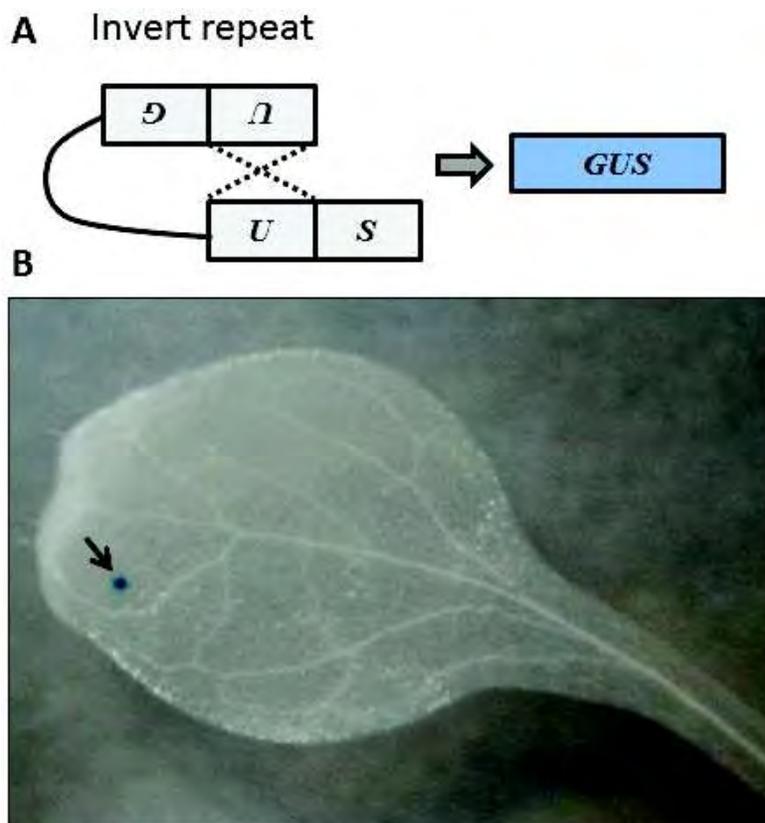

Fig. 1: **(A) Homologous recombination event in plants leading to complete *GUS* gene restoration [11]. (B) Blue spots seen after reversion of *GUS* into its functional form**
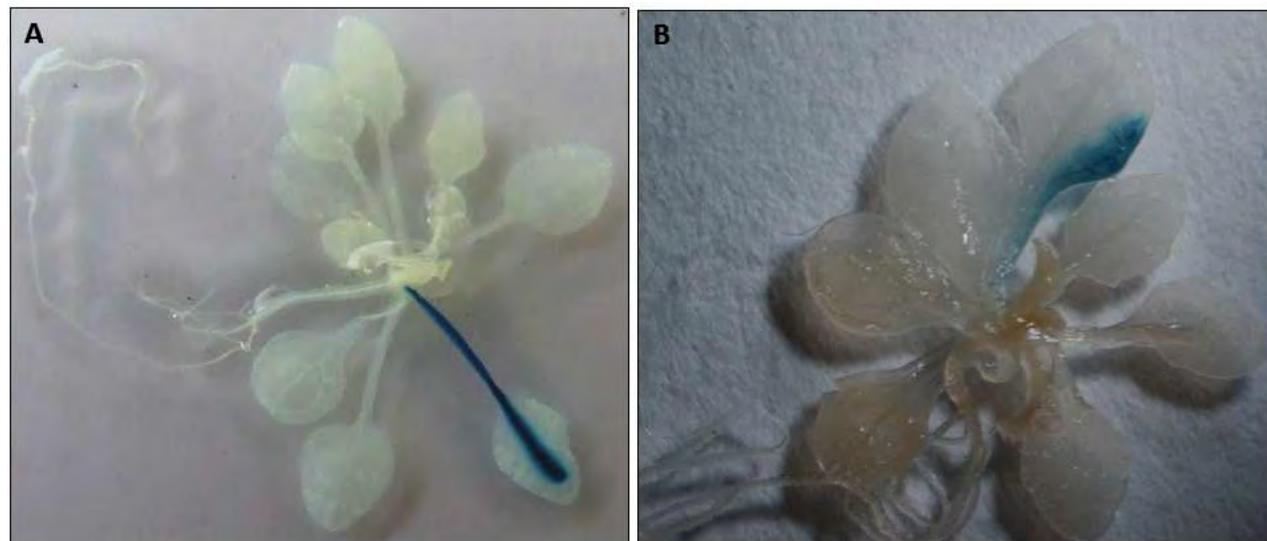
Fig. 2: *Arabidopsis* **plants harboring CaMV 35S promoter-driven** *GUS* **gene mutated for detecting spontaneous somatic homologous recombination. Spontaneous reversion of mutated** *GUS* **into functional form is displayed by the blue colored tissue, derived from the same progenitor cell, after GUS histochemical staining. (A) Petiole and midrib. (B) Parts of leaf lamina**

stained plant material was very less and hence we could not perform other genomic studies. However, plants from the same line have been used previously [11, 18] where, Southern blot analyses have revealed that the blue region indeed is due to homologous recombination in the inverted *GUS* construct.

Since the frequency of spontaneous recombination is very low (10^{-6} events per cell division) [18], we propose that HR in the *GUS* gene had taken place in one of the cells of the leaf primordium and, petiole and midrib generated from this particular cell. Also, if they were descendents of a group of cells from the primordium, then they would not have uniformly stained blue. Due to the very low rate of spontaneous somatic recombination, we did not obtain the same example again. Although, using these mutated *GUS* lines were meant to screen point mutations or HR, we did obtain many other similar examples which reflected the plant developmental process (Fig. 2B), which were previously known [3].

We did observe similar results in plants with *GUS* gene interrupted with a microsatellite [1]. We did not consider that data because the frequency of

such frame-shift mutations was very high (about 6.5 blue spots per leaf). Due to this, it could be that more than one adjacent cell could undergo mutation and give the blue colour, and that would be a misleading interpretation of the plant developmental anatomy.

This is the first report revealing the most probable origin of petiole and midrib from leaf primordium and since there is a lack of easily identifiable markers of cell states preceding procambium formation [16], it is important to pin down such events that reveal plant developmental secrets.

Acknowledgements

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