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1 **VIP1 is Very Important/Interesting Protein 1 regulating touch responses of Arabidopsis**

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13

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16 **Key words:** *Arabidopsis thaliana*; bZIP transcription factor; calcium signaling; cell death;

17 cell wall; hypo-osmotic stress; mechanical stimuli; protein phosphorylation; root cap; root

18 waving

19

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22 **Abstract**

23 VIP1 (VIRE2-INTERACTING PROTEIN 1) is a bZIP transcription factor in *Arabidopsis*
24 *thaliana*. VIP1 and its close homologs (i.e., Arabidopsis group I bZIP proteins) are present in
25 the cytoplasm under steady conditions, but are transiently localized to the nucleus when cells
26 are exposed to hypo-osmotic conditions, which mimic mechanical stimuli such as touch.
27 Recently we have reported that overexpression of a repression domain-fused form of VIP1
28 represses the expression of some touch-responsive genes, changes structures and/or local
29 auxin responses of the root cap cells, and enhances the touch-induced root waving. This
30 raises the possibility that VIP1 suppresses touch-induced responses. VIP1 should be useful to
31 further characterize touch responses of plants. Here we discuss two seemingly interesting
32 perspectives about VIP1: (1) What factors are involved in regulating the nuclear localization
33 of VIP1?; (2) What can be done to further characterize the physiological functions of VIP1
34 and other Arabidopsis group I bZIP proteins?

35

36 **TEXT**

37 VIP1 (VIRE2-INTERACTING PROTEIN 1) was originally identified as an *Arabidopsis*
38 *thaliana* bZIP protein interacting with the *Agrobacterium tumefaciens* protein VirE2,¹ and has
39 been suggested to play pleiotropic roles.¹⁻¹² Previously we reported that VIP1 exists mainly in
40 the cytosol under steady conditions but transiently accumulates in the nucleus when cells are

41 exposed to hypo-osmotic conditions,⁹ that close homologs of VIP1 (i.e., Arabidopsis group I
42 bZIP proteins¹³) also exhibit such a pattern of subcellular localization,¹¹ and that overexpression
43 of a repression domain-fused form of VIP1 (VIP1-SRDX) enhances touch-induced root
44 waving.¹⁴ Touch as well as the hypo-osmotic conditions seems to induce the nuclear
45 localization of VIP1 (Fig. 1). VIP1 is a novel regulator of touch responses of Arabidopsis,
46 and should be useful to further characterize them. Here we discuss perspectives for further
47 studies on VIP1.

48

49 **What factors are involved in regulating the nuclear localization of VIP1?**

50 Previously a treatment with a microbe associated molecular pattern, flg22, caused VIP1 to be
51 localized to the nucleus,⁶ and a treatment with a gibberellin biosynthesis inhibitor, uniconazole-P,
52 caused the putative tobacco VIP1 ortholog RSG (REPRESSION OF SHOOT GROWTH) to
53 be localized to the nucleus.¹⁵ However, in our experiments, water itself, which was used to
54 dilute flg22 and uniconazole-P stocks in those experiments, can induce the nuclear localization of
55 VIP1 (ref. 10 and unpublished data), thus the effects of these chemicals may have to be
56 re-evaluated.

57

58 VIP1 is thought to accumulate in the nucleus when its 79th serine is phosphorylated by
59 MPK3 (MITOGEN-ACTIVATED PROTEIN KINASE 3),⁶ although this idea has been

60 questioned.¹¹ On the other hand, RSG is thought to accumulate in the nucleus when its
61 phosphorylated 114th serine is either dephosphorylated or replaced by alanine.¹⁵ Although
62 replacing the VIP1 115th serine, which corresponds to the RSG 114th serine, with alanine
63 does not affect the subcellular localization of VIP1,¹¹ our unpublished data suggest that VIP1
64 has multiple putative phosphorylation sites, and that these sites are dephosphorylated when
65 VIP1 is localized to the nucleus. The RSG 114th serine is phosphorylated by the
66 calcium-dependent protein kinase CDPK1 in tobacco,¹⁶ thus a CDPK1 homolog may
67 phosphorylate VIP1 in Arabidopsis. Our unpublished data also suggest that calcium signaling
68 regulates both the nuclear import and the nuclear export of VIP1. It should be interesting to
69 identify protein kinases and protein phosphatases regulating VIP1 phosphorylation states, and
70 to identify the phosphorylation sites in VIP1.

71

72 The mechanosensitive calcium channels MCA1 and MCA2 (MID-COMPLEMENTING
73 ACTIVITY 1 and 2, respectively), the seven-transmembrane domain proteins MLO4 and
74 MLO11 (MILDEW RESISTANCE LOCUS O 4 and 11, respectively), and the receptor
75 kinase FERONIA have been identified as candidate mechanical stress sensors regulating
76 calcium signaling and root tropisms.¹⁷⁻²⁰ *TOUCH2* and *TOUCH4*, which are up-regulated by
77 the FERONIA-mediated touch-responsive signaling,²⁰ are unlikely to be VIP1 target genes.¹⁴
78 However, it would be worth examining whether VIP1 interacts with these proteins and/or acts

79 under the control of them. To identify novel regulators for VIP1, it would also be helpful and
80 practical to screen for chemicals and genetic mutations that affect the subcellular localization
81 of VIP1.

82

83 **What can be done to further characterize the physiological functions of VIP1 and other**
84 **Arabidopsis group I bZIP proteins?**

85 VIP1 and its close homologs in tomato, tobacco, and rice, have been identified in different
86 studies as a regulator of certain physiological responses.^{15,16,21-26} This would support the idea
87 that such VIP1 homologs have important, pleiotropic roles. Thus far only two Arabidopsis
88 group I bZIP protein genes, *VIP1* and *PosF21*, have been associated with physiological
89 roles.^{1-12,14,27} However, at least five of the other group I bZIP protein genes are expressed as
90 highly as *VIP1* and *PosF21*,¹¹ and they could function redundantly. In our recent study,
91 expressing the VIP1-GFP fusion protein suppressed the VIP1-SRDX-induced enhancement
92 of root waving.¹⁴ It would be interesting to examine whether other group I bZIP proteins can
93 also suppress the VIP1-SRDX-induced enhancement of root waving. In our preliminary
94 experiments, the triple mutant that has T-DNA in *VIP1*, *PosF21*, and *bZIP29* (another group I
95 bZIP protein gene) was similar to the wild type in phenotypes including root waving, but
96 knocking out various combinations of the group I bZIP protein genes should also help to
97 elucidate the physiological roles of them.

98 The local auxin responses in the root tip are different between wild-type plants and
99 VIP1-SRDX-overexpressing (VIP1-SRDXox) plants, and this may be attributed to the
100 difference in adhesion and/or removal of the root cap cells. Expression levels of some
101 mechanical stimulus-induced genes that should regulate cell wall properties are lower in
102 VIP1-SRDXox plants than in the wild-type plants, and this may cause the abnormal root cap
103 cell adhesion/removal in VIP1-SRDXox plants.¹⁴ On the other hand, cell death mediated by
104 the NAC (NO APICAL MERISTEM)-family transcription factor SOMBRERO and the S1-P1
105 nuclease-family protein BFN1 (BIFUNCTIONAL NUCLEASE 1) is necessary for the
106 removal of the lateral root cap cells.^{28,29} It would be interesting to characterize the cell wall
107 properties and cell death in the root cap cells of VIP1-SRDXox plants, and to examine
108 genetic and physical interactions between the group I bZIP proteins and the above regulators
109 of cell wall properties and cell death. It would also be important to further evaluate how
110 mechanical stimuli affect cell wall properties and cell death in root cap cells.

111

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197

198 **Figure Legend**

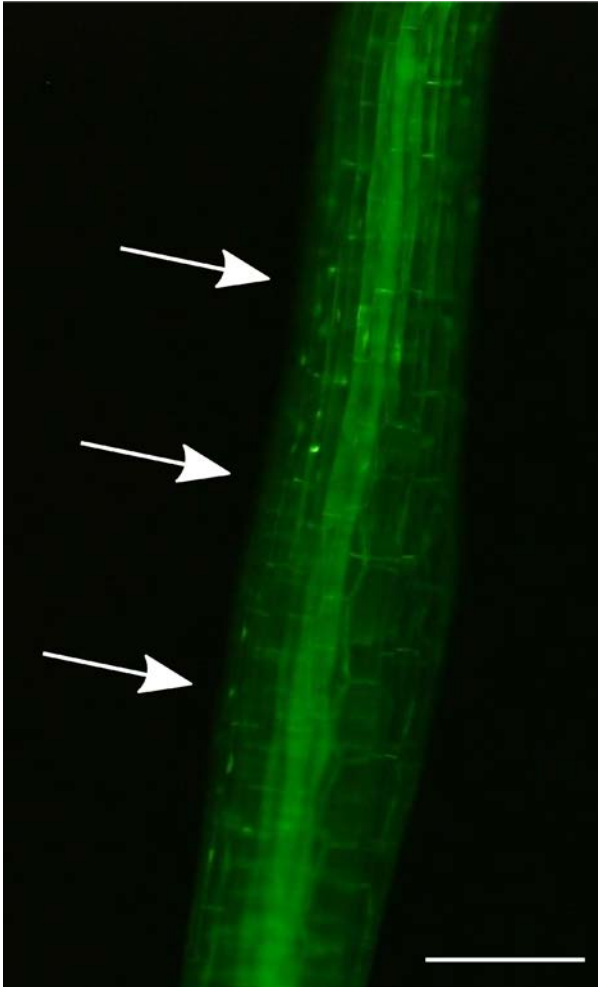
199 **Figure 1.**

200 **Touch-induced nuclear localization of VIP1.**

201 Transgenic Arabidopsis plants expressing the VIP1-GFP fusion protein were grown for 3 days in
202 darkness. VIP1-GFP signals were observed approximately 3 minutes after their hypocotyls
203 were touched by a needle tip. More than five plants were used, and a representative result is
204 presented. Arrows indicate the approximate position where the hypocotyl was touched.

205 Scale bar = 200 μ m.

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