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Research Note

A putative NAP homolog specifically expressed during grapevine flower and berry development

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Introduction: In flowering plants, fruit morphogenesis is a distinct and unique process following fertilization. Most fleshy fruits as grapevine develop from ovary tissues (Eames and MacDaniels 1947) and exhibit convergent characteristics such as pericarp cell proliferation and enlargement. The early molecular events that control the dynamics of fruit morphogenesis remain obscure. From preliminary analysis of the fleshless berry mutant (*Vitis vinifer*a L. cv. Ugni Blanc) which is unable to specify ovary cell fate later in fleshy pericarp development, we have identified a gene homolog of *PISTILLATA* up-regulated in the mutant ovary. In *Arabidopsis*, *NAP* (AJ222713) appeared as an immediate target of the floral homeotic genes *PISTILLATA/APETALA3* (Slablowski and Meyerowitz 1998). *NAP* genes belong to the NAC domain gene family encoding transcriptional regulators required for meristem establishment and other plant developmental processes. The original genes *NO APICAL MERISTEM* (*NAM*) from petunia, *ATAF1,* 2 and *CUP-SHAPED COTYLEDON* (*CUC2*) from *Arabidopsis* defined the highly conserved NAC protein domain (Aida *et al.* 1997). We report, here, quantitative expression patterns of the closest *NAP* grapevine homolog (TC38700 from the TIGR database), *VvNAP*, during grapevine development of the *flb* mutant and the wild type genotypes.

Material and Methods: The *flb* mutation was identified in 1996 in a vineyard located at Prades-le-Lez (France) on a *Vitis vinifera* cv. Ugni-Blanc anticlinal chimeric plant bearing some shoots with berries dramatically reduced in size. For *flb* mutant and wild-type Ugni Blanc, samples corresponded to inflorescences from stage 17 (mature inflorescence with all flowers formed and separated) and 19 (first flower caps loosening) of the E-L system (Coombe 1995) and berries from 0 to 20 d after anthesis collected in the greenhouse (ENSA.M-INRA, Montpellier). For *V. vinifera* cv. Cabernet-Sauvignon, samples corresponded to vegetative organs (young roots, shoot tips including 2 to 3 expanded leaves and young leaves), inflorescences from stages 7 (immature inflorescence emerging from buds) and 17, flowers at flowering time (50 % caps off) and seedless berries (2-16 weeks post flowering), harvested in the field (Clare Valley, Australia).

Total RNA was extracted using RNeasy Plant Mini kit (Qiagen) for Ugni Blanc and according to Rezaian and Krake (1987) with slight modifications as described in Boss *et al.* (2001) for Cabernet-Sauvignon.

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After DNase treatment (RNase-Free DNase Set, Qiagen), reverse transcription of 1 µg total RNA was carried out using Superscript III reverse transcriptase (Invitrogen) with a 20mer-dT primer. Gene specific primer pairs were designed to amplify 150-250 bp in the 3'end of the *VvNAP* (TC38700) (F_ CTTAGCTCGGTTGCGGTTAC, R_ CTAGCGCTCTTCCCTGACAA), *VvUBI* (CF406001) (F_AGTAGATGACTGGATTGGAGGT, R_GAGTATCAAAACAAAAGCATCG) and *Vvef1alpha* (BQ799343) (F_GAACTGGGTGCTTGATAGGC, R_AACCAAAATATCCGGAGTAAAAGA) genes.

PCR amplifications were performed from 1 µl of 1/10 diluted ss cDNA, using the SYBR Green PCR Master Mix (Perkin -Elmer, Applied Biosystems) with a Rotor-Gene 2000 (Corbett Research, NSW, Australia) or a 7700 Sequence Detection System (Applied Biosystems, Warrington, UK) apparatus. Quantification was obtained by plotting the cycle threshold value against the linear calibration curve obtained with serially diluted cDNA of the target gene. Sample values were corrected using the corresponding expression level of the control genes (*VvUBI* and *Vvef1alpha)* and expressed as the relative abundance ± SD.

Results and Discussion: Soon after fertilization the fleshless berry (*flb*) mutation inhibits the development of the mesocarp resulting in a pericarp reduced in size by approximately 20-fold at maturity. The *flb* mutant and its wild-type counterpart were previously used to screen genes potentially involved in early fruit morphogenesis. Among genes up-regulated in the mutant, a transcript coding for a PISTILLATA homolog was identified through suppression subtractive hybridizations (data not shown). This factor was shown to activate the expression of *NAP* that was suggested to function in the transition between growth by cell division and cell expansion in *Arabidopsis* floral tissues (Slablowski and Meyerowitz 1998). Search for *NAP* (AJ222713) similarities through tblastn programs in the grapevine TIGR database identified the TC38700 contig (*VvNAP*) showing a high degree of similarity in the NAC protein domain (Fig. 1). In the *flb* mutant, *VvNAP* expression exhibited a constant over-expression compared to the wild-type, in both flower and young fruit organs (Fig. 2 A), which was compatible with *PISTILLATA* over-expression observed in this genotype (data not shown). We supposed that *PISTILLATA* activated *VvNAP* expression in grapevine, as observed for *NAP* in *Arabidopsis*. No expression of *VvNAP* was found in grapevine vegetative organs such as leaves, shoots or roots (Fig. 2 B), also similar to *NAP* in *Arabidopsis* (Slablowski and Meyerowitz 1998). Significant expression was detected in mature inflorescence, flower and in fruit at any stage of development, following a bimodal profile with a maximum of expression level at stage of complete ripening (Fig. 2 B). The present results appeared somewhat paradoxical since up-regulation in the mutant suggested a negative effect of *VvNAP* on ovary development, while its specific expression in reproductive organs suggested an ongoing role on flower and fruit development. Over-expression of *VvNAP* could be a distant consequence of the *flb* mutation and, in this case, is possibly not directly correlated to the mutant phenotype. Alternatively, a defined level of VvNAP may be necessary for proper

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AtNAP:      6  QSTLPPGFRFHPTDEELIVYYLRNQTMSKPCPVSIIPEVDIYKFDPWQLPEKTEFGENEW
Consensus:     Q  LPPGFRFHPTDEEL V YL     S P PV II EVD YKFDPW LP K  FGE EW
VvNAP:     12  QPQLPPGFRFHPTDEELVVHYLKKKASSAPLPVAIIAEVDLYKFDPWELPAKASFGEQEW

AtNAP:         YFFSPRERKYPNGVRPNRAAVSGYWKATGTDKAI--HSGSSNVGVKKALVFYKGRPPKGI
Consensus:     YFFSPR RKYPNG RPNRAA SGYWKATGTDK      G   VGVKKALVFY G PPKGI
VvNAP:         YFFSPRDRKYPNGARPNRAATSGYWKATGTDKPVLTSGGTQKVGVKKALVFYGGKPPKGI

AtNAP:         KTDWIMHEYRLHDSRKASTKRNG--------SMRLDEWVLCRIYKK 161
Consensus:     KT WIMHEYRL D  K  TK  G        S RLD WVLCRIYKK
VvNAP:         KTNWIMHEYRLADN-KVNTKPPGCDMGNKKNSLRLDDWVLCRIYKK 176
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Fig. 1: Alignment of the NAC proteic domain, an N-terminal module of ~160 amino acids, of VvNAP (from TC38700, 353 amino acids) and AtNAP (CAA10955, 268 amino acids). Numbers corresponded to the NAC domain position in the protein.

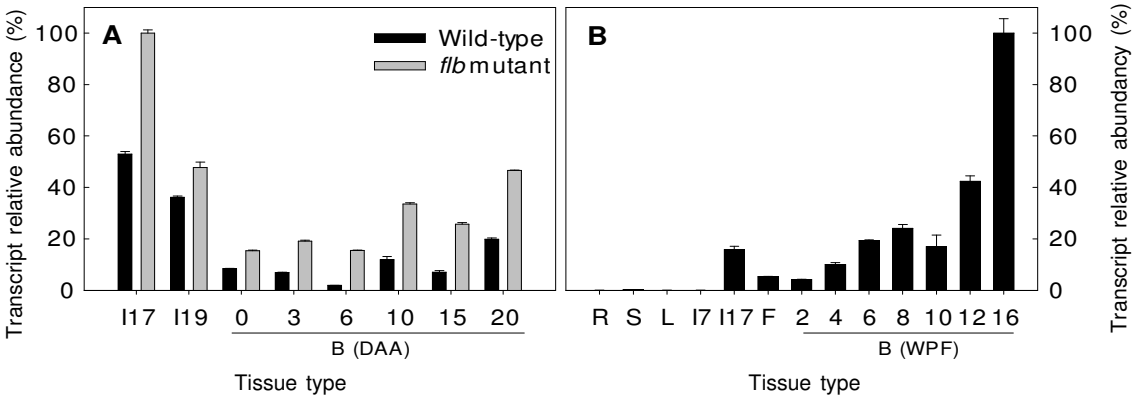

Fig. 2: Real time PCR expression profiling of *VvNAP*. **A**: Expression in the *flb* mutant and the wild-type: inflorescences from stage 17 (I17) and 19 (I19) of the E-L system (COOMBE 1995) and berries (B) at 0, 3, 6, 10, 15 and 20 d after anthesis (DAA). **B**: Expression in Cabernet Sauvignon grapevine organs and at various developmental stages: roots (R), shoots (S), leaves (L), inflorescences at stages 7 (I7) and stage 17 (I17), seedless berries at 2, 4, 6, 8, 10, 12, 16 weeks post flowering (WPF). Expression values have been normalized with *Vvef1á* and *VvUBI* in **A** and **B** respectively (bars = SD of PCR technical triplicates).

growth, as was considered for NAP since in its absence, cells cannot shift to the elongation mode, and with sustained expression, the transition between cell division and expansion can not be finished (SLABLOWSKI and MEYEROWITZ 1998). No expression of *NAP* was reported in the *Arabidopsis* silique and the role of *NAC* domain genes in fruit development remains poorly documented. In grapevine berries, expression of some members of the *NAC* gene family have been reported (TERRIER *et al*. 2005). Although initial studies focused on the SAM function of *NAC* genes, it is increasingly clear that the *NAC* domain genes in the *Arabidopsis* genome represent a broad class of regulatory genes unique to plants that functions in controlling diverse plant functions and probably inducing non-meristematic functions such as hormonal control and defense (DUVAL *et al.* 2002). We report here expression data about *VvNAP,* the putative *Arabidopsis NAP* ortholog, possibly important for grapevine flower and fruit development. Additional experiments such as *in situ* localization and specific gene silencing studies would be necessary to determine the precise function of this gene in grapevine.

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