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Over Expression of *KNAT1* Gene in Phalaenopsis Orchid Keeps the Plant Cells Under Meristematic Condition

Rindang Dwiyani*

Faculty of Agriculture, Udayana University, Bali, Indonesia

Short Communication

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***For Correspondence**

Rindang Dwiyani, Faculty of Agriculture, Udayana University, Bali, Indonesia, Tel: 0361702801

E-mail: rindangdwiyani@yahoo.co.id

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ABSTRACT

*KNAT*1 transformant of *P. amabilis* orchid was produced and subsequently it able to produce a greater number of propagules and plantlets via micropropagation compared to those of the wild type. It indicated that Overexpression of *KNAT1* Gene keeps the plant cells under meristematic condition

Genes of *Knotted-Like from Arabidopsis thaliana* (*KNAT*) comprises of *KNAT1, KNAT2 and KNAT6* and are grouped as Class 1 *KNOX* genes in *Arabidopsis thaliana (A. thaliana)* [1]. The KNAT genes with other KNOX gene i.e. *SHOOTMERISTEMLESS (STM)* gene, keep shoot apical meristem (SAM) in the meristematic state [2,3]. STM gene is active during the vegetative growth of *A. thaliana* and keeps stem cell pools in the SAM. However, the role of *STM* in keeping stem cell pools in the SAM can only be substituted by overexpression of *KNAT1* gene, but not with other KNOX genes [3].

A previous research found that micropropagation of *Phalaenopsis amabilis* (*P. amabilis*) and *Vanda tricolor* orchids carrying *KNAT1* gene resulted in a greater number of buds compared to its wild type (WT), indicating that *KNAT1* gene expressed in the organ of transformants, keeping cells at 'meristematic state' (similar to SAM) as they easily regenerated and formed buds [4].

The role of *KNAT1* gene in keeping plant cells under meristematic state also present on protocorms that carrying the gene. Protocorms of *P. amabilis* orchid carrying *KNAT1* gene produced up to 35 shoots, while WT only produced one to three shoots [5]. Protocorm is a globular-green or yellow structure that formed when orchid seed germinates.

KNAT1 transformant of *P. amabilis* was produced and subsequently it able to produce a great number of plantlets via micropropagation, however, proliferation did not occurred in the WT plants. Cytokine may still require inducing bud formation. As seen in **Figure 1**, a shoot transverse section of *KNAT1* transformant of *P. amabilis* produced more buds when planted on new phalaenopsis (NP) medium with addition of 5 µM 2-iP (2-isophentenyladenine) and 0.15 µM NAA (Naphthalene Acetic Acid) compared to that of NP medium only [6].

The application of cytokines such as 2-iP might change the status of indeterminate state of those meristematic cells in the organ into determinate state, so they formed buds, roots and then grew to be plantlets [4].

All organs of the *KNAT1* transformant produced buds after 9 weeks planting on NP medium added with 5 µM 2-iP (2-isophentenyladenine) and 0.15 µM NAA (Naphthalene Acetic Acid) except leaf tip. Leaf base, root base and transverse section of shoot produced greater number of buds, indicated that these part of organs were more meristematic compared to others. However, it needs further research to explain why these parts of plant were more meristematics compared to other organs in the *KNAT1* transformant.

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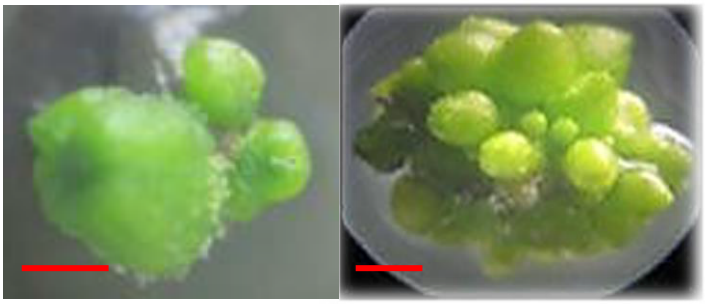

Figure 1. Development and growth of shoot transverse section from *KNAT1* trasformant planted on NP medium only (left) and NP medium with addition of cytokine (right). Bar=5 mm.

Various phenotypes were determined in the progenies resulted from micropropagation of *KNAT1* transformant of *P. amabilis* as seen in **Figure 2**, indicated that somaclonal variation occurred. Although the source of explants was a plant with normal phenotype, however, abnormal phenotypes were found in the progenies. For example, some plantlets had rosette leaves (b, f) and some had upright growing-roots (d, e, h, i, j), however, there were normal plantlets (k, l, m) were also produced. Somaclonal variation was frequently occurred in micropropagation and has been reported on Heliconiaceae [7], strawberry [8], sugarcane [9] and others. The study [4] was also investigated that using PCR analysis with specific primer for *KNAT1* gene, the *KNAT1* gene was derived in to some progenies.

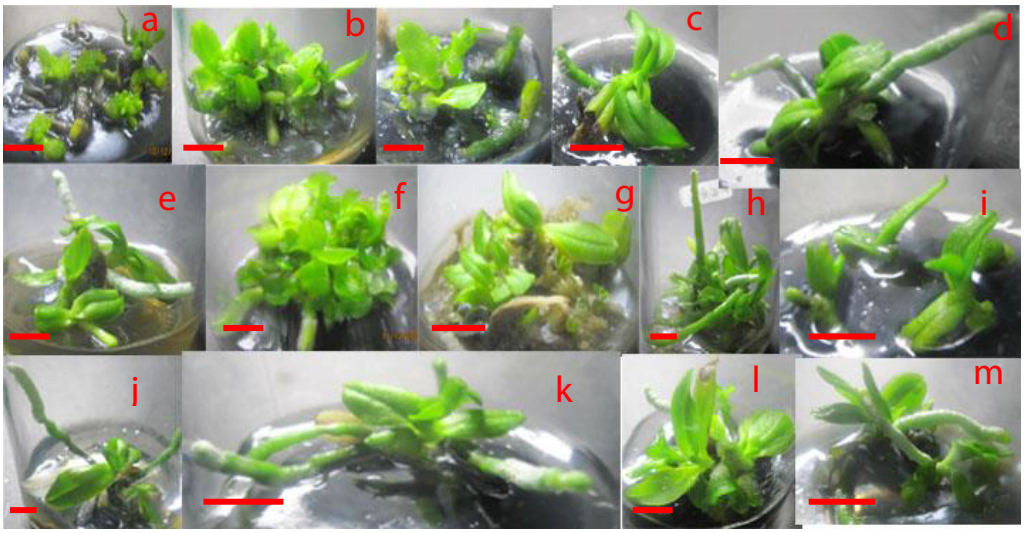

Figure 2. Profiles of plantlet from explants of root base of KNAT1 transformant of *P. amabilis* at 15 weeks after planting; Abnormal phenotypes of rosette leaves (b,f) and upright growing-roots (d, e, h, i, j) were detected. Bar=1 cm.

Three points can be concluded from the study [4]. First, Overexpression of *KNAT1* Gene in *P. amabilis* orchid keeps the plant cells under meristematic condition. Transformant plants produced more buds compared to WT; Second, various phenotypes were determined in the progenies included abnormal plantlets although plant with normal phenotype was used as source of explant; Third, *KNAT1* gene was still detected in the progenies

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