Take control over horticulture by listening to the genes©

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INTRODUCTION

Long before changes in the condition of cultured plants become visible, changes on the gene activity level already occurred; changes that originate from varying climate conditions or infection by pathogens. Also changes provoked by horticultural measures, on the climatological level, by nutrition, or application of agrochemicals.

NSure is a company specialised in detecting early changes in the activity of genes related to specific traits. NSure proved that analysis of these early changes adds value to decision support systems. One can act early and before it is too late. In what follows, a few examples of applications are being discussed in order to illustrate how this approach functions in practice. But first, some background information about the methodology is given.

METHODOLOGY

To get insight in changes in gene activity, NSure applies so-called Next Generation Sequencing (NGS). This technology results in knowing what genes are active at a certain moment and to what extent they are active. By comparing for instance plants that have or have not been treated with certain agents, one can investigate which metabolic pathways are being triggered as a result of this specific treatment. Upon considering that plants are ever changing during their lifecycle, one needs a sound trial design to make the correlation and draw the right conclusion. That is where expert knowledge becomes a prerequisite. Based on experience three different focus areas have been defined where the technology adds value. The first focus is on physiological switches. Early recognition of cold tolerance, bud break potential and exact ripening stage helps stakeholders to meet logistic challenges and optimise yields. The second focus point is early warning. First signs of upcoming diseases, before symptoms occur, can be recognised and harvests can be saved by acting on the knowledge gained. The third focus area of NSure is the so-called bioresponse. Effects of for instance biostimulants are being studied in detail. Insight in the mode of action of these agents is obtained and product claims supported. Moreover, once it is known what genes are most affected by the applied agent, activity measurements of such genes can assist optimisation of the application. Different formulations or dosages can be easily and quickly compared, application and reapplication moments can be optimised.

Regarding the latter, there is a connection with the focus area physiological switches. Maximal efficacy often depends heavily on the actual stage of the plant. Detailed insight in such a relevant stage increases the success rate of the application.

Once gene activity indicators are being found via NGS, their activity can be measured routinely by PCR. This method is focused on activity measurements of single genes and is more cost effective in comparison to before mentioned NGS. PCR-based measurement of gene activity for a pre-defined set of genes, the indicators, is called a molecular test.

EXAMPLES

In what follows two molecular tests are being highlighted: the ColdNSure and the recently released BreakNSure. In addition, an example of a mode of action study is described.

The ColdNSure test is being used by nursery managers producing Pine and Spruce seedlings. The users are situated mainly in Scandinavia. In autumn, at the end of the growing

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season, one- or two-year-old seedlings become dormant and frost tolerant. At a certain moment, seedlings are being packed and put in frozen storage until next spring. Then they are distributed and planted at their final location. Seedlings cannot be stored in frozen storage until they are fully hardened. Otherwise, upon replanting, severe losses are being observed.

By using the ColdNSure test one can get certainty about full frost-tolerance. As can be seen in Figure 1A, the switch from frost sensitive to frost tolerant is realised within a single week. This switch can be recognised in the activity pattern of certain genes (Figure 1B). By measuring the activity of these genes, one can conclude whether a given batch of seedlings is ready for storage or not yet (Stattin et al., 2012).

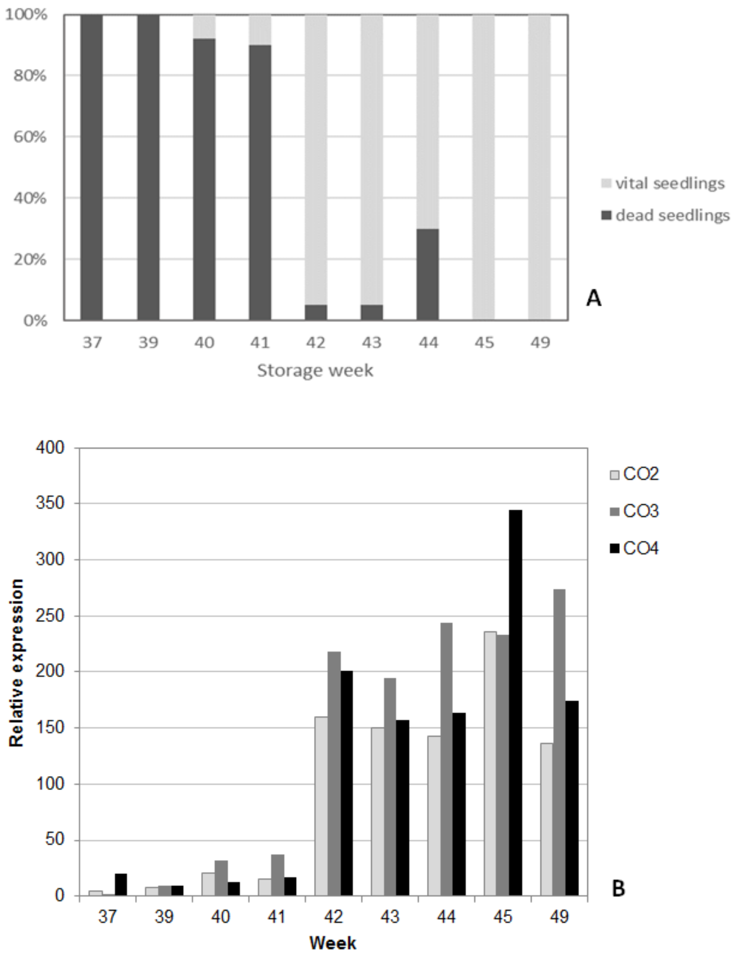
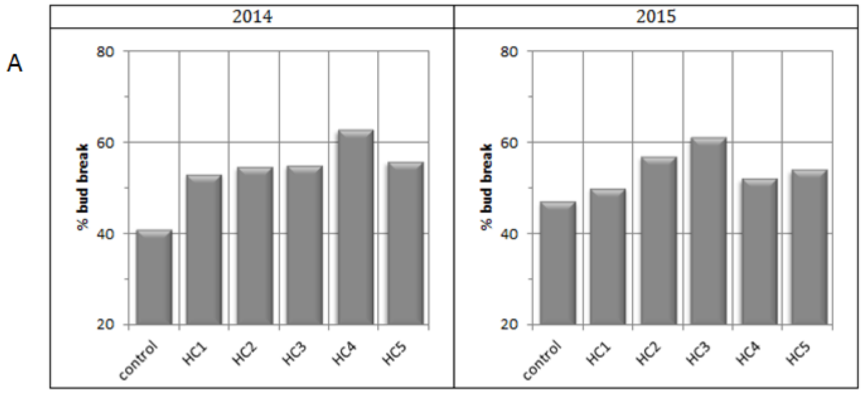


Figure 1. A: Vitality of *Picea abies* seedlings after storage and upon replanting in relation to the moment of transfer to frozen storage. From Week 41 on, seedlings can be stored safely. B: A clear switch in the activity of specific genes coincides with the indicated moment where seedlings can be transferred safely.

The BreakNSure test is meant to define the proper moment to apply bud break enhancing agents in kiwifruit (*Actinidia*) production. Maximal and synchronous flowering is gained by the application of such agents but only when the vines are in the right stage. Gene activity measurements assist in defining the proper stage. Figure 2A shows that the optimal moment of application varies, in this case between the years. Suboptimal application results in a considerably lower number of bud break, flowers, and subsequently fruit. A gene index, based on activity measurement of carefully selected genes, can be used to advise the proper moment of application (Figure 2B). Since 2016 this test is being used for the application of HiCane by Zespri growers in New Zealand. At this moment NSure is evaluating the use of the test for optimisation of the application moment of alternative bud break enhancing agents, both in Italy and New Zealand (Hoeberichts et al., 2017). In addition, the BreakNSure test is being developed for other fruit, including sweet cherry and table grape (Balk et al., in press).



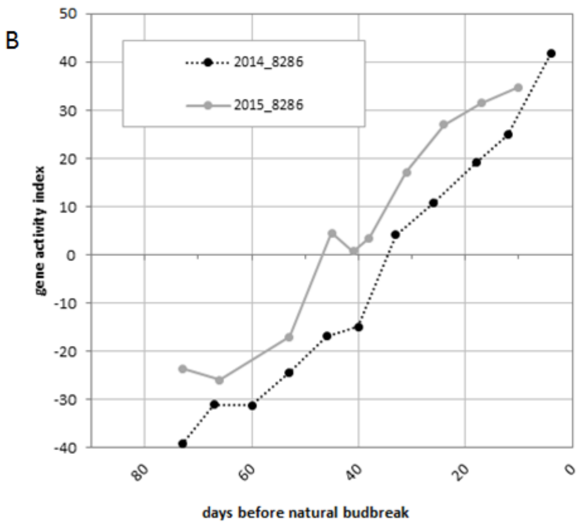


Figure 2. A: HiCane application at five subsequent moments results in increased percentages of bud break. However, a distinct optimal moment of application can be observed: moment 4 in 2014, moment 3 in 2015. B: By using a gene activity index, one can define a range of scores where HiCane can be applied with optimal result.

Any of NSure's molecular tests consists of two steps, sampling and analysis. The easy sampling method developed by NSure is can be used on location and is done by the customer. Analysis of the collected sample is performed at a nearby laboratory. Results are delivered within 2 working days.

Gene activity measurements can also be applied for gaining insight into the mode of action of, for example, a biostimulant. This particular biostimulant enhances wound healing in tomato plants after deleafing (Figure 3A). The risk of infections is reduced by stimulated scar tissue formation. Already 6 h after application, considerable changes in gene activity were observed. The nature of these genes point towards the direction of an enhanced abscisic acid (ABA) mediated response. Figure 3B shows three of such genes, all involved in the well described ABA response. Scientific evidence for stimulation of scar tissue formation mediated by ABA (Leide et al., 2012) in combination with the gene activity data provides evidence that this specific biostimulant indeed promotes wound healing via an enhanced ABA mediated response.

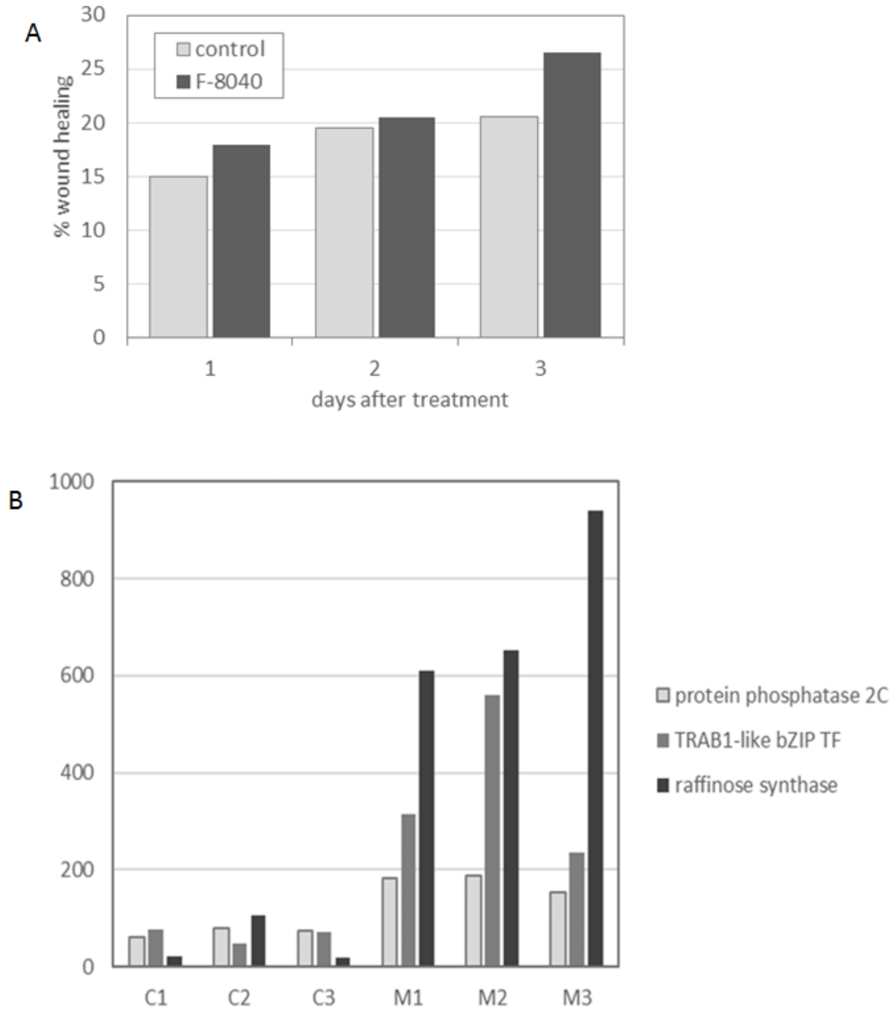


Figure 3. A: The Biostimulant F-8040 stimulates wound healing in tomato. Shortly after application positive effects can be observed. B: Among numerous affected genes, several related to the response to abscisic acid were observed. The activity of three such genes are displayed here in triplicate samples (C = Control, M = Treated with F-8040).

CONCLUSION

Focus on gene activity provides insight into what is going on inside a plant in preparation of changing performance. The above examples show that activity measurements of specifically selected genes may assist agricultural practice. Production is optimised and spoilage can be actively reduced. Gene activity measurements therefore fit perfectly well in a more sustainable agricultural practice: know when to act and at the earliest stage.

Literature cited

Balk, P.A., Hoeberichts, F.A., Verhoef, N., Schumacher-Strijker, A.M., and Aanhane, T.G.M. Monitoring dormancy release in fruit trees and ornamentals by RNA Sequencing and its implications for horticulture. Acta Hortic. (in press).

Hoeberichts, F.A., Povero, G., Ibanez, M., Strijker, A., Pezzolato, D., Mills, R., and Piaggesi, A. (2017). Next generation sequencing to characterise the breaking of bud dormancy using a natural biostimulant in kiwifruit (*Actinidia deliciosa*). Sci. Hortic. (Amsterdam) *225*, 252–263 https://doi.org/10.1016/ j.scienta.2017.07.011.

Leide, J., Hildebrandt, U., Hartung, W., Riederer, M., and Vogg, G. (2012). Abscisic acid mediates the formation of a suberized stem scar tissue in tomato fruits. New Phytol. *194* (*2*), 402–415 https://doi.org/10.1111/j.1469-8137.2011.04047.x. PubMed

Stattin, E., Verhoef, N., Balk, P., Wordragen, M., and van Lindström, A. (2012). Development of a molecular test to determine the vitality status of Norway spruce (*Picea abies*) seedlings during frozen storage. New For. *43* (*5-6*), 665–678 https://doi.org/10.1007/s11056-012-9320-1.