OXFORD

Briefings in Functional Genomics, 15(1), 2016, 10–15

doi: 10.1093/bfgp/elv008
Advance Access Publication Date: 29 March 2015
Letter to the Editor

Calorespirometry, oxygen isotope analysis and functional-marker-assisted selection ('CalOxy-FMAS') for genotype screening: A novel concept and tool kit for predicting stable plant growth performance and functional marker identification

Birgit Arnholdt-Schmitt, Lee D. Hansen and Amaia Nogales

Corresponding author. Birgit Arnholdt-Schmitt, EU Marie Curie Chair – ICAAM, IIFA, Universidade de Évora, Pólo da Mitra, Apartado 94, 7002-554 Évora, Portugal. Tel.: +351 266760800; E-mail: eu_chair@uevora.pt

Abstract

We propose a novel concept and tool kit for predictive phenotyping. The proposed technology measures respiration properties as functions of growth conditions to identify genotypes with higher plasticity via homeostasis and adaptive morphophysiology. Combining calorespirometry, oxygen isotope analysis and functional-marker-assisted selection ('CalOxy-FMAS') for genotype screening will enable predicting the genetic potential for stable plant growth performance. Application of this novel tool kit can help identify genotypes with controlled homeostasis in changing environments and optimized growth performance. Simultaneously, it will allow a better balance in breeding for high yields and quality characteristics. Applying 'CalOxy-FMAS' can efficiently narrow the pool of genotypes to be screened for final phenotyping in the field.

Key words: emerging technologies; functional marker development; deep phenotyping; plant breeding; calorespirometry

The problem

Plant breeders need to identify appropriate genotypes to build-up improved breeding populations and finally varieties that can provide farmers with predictable and stable plant production. However, major parameters for tracing stable plant growth performance as a trait and for simplified selection have not been identified.

Functional genomics aims to assist conventional breeding by providing molecular tools to allow more efficient genotype selection for several important agronomic traits such as stress tolerance. However, a large gap still remains between good intentions, much effort and investments, and growing insights into biological complexity, and effective and stable improvement of genetic material. This gap is especially obvious where

Birgit Arnholdt-Schmitt is Coordinating Investigator and head of the EU Marie Curie Chair, at the University of Évora. She coordinates the Competence Focus 'AOX Research and Functional Marker Development'. The ideas for this proposal were highly inspired by working together with the company INOQ GmbH (Germany) on the holobiont concept within the European Industry—Academia Partnership and Pathway project AGRO-AMF-AOX and the COST Action FA1103.
Lee D. Hansen is a Professor Emeritus of Chemistry and Biochemistry at Brigham Young University in Provo, Utah, USA, with many years of experience in applying calorimetry and thermodynamics to biology.
Amaia Nogales is postdoc scientist at the EU Marie Curie Chair, and she was trained in a 3-years project in calorespirometry. She leads now a project focused on calorespirometry as a tool to study cold stress response in mycorrhizal plants. She has experience also on AOX and COX *in vivo* activity measurements.

general robustness of plants, which requires multi-stress tolerance, is concerned. The scientific community of today cannot cope with the huge amount of 'omics' data from stress research, a difficult problem in data mining. But the more important problem may lie in the fact that knowing all the details of genetic and metabolic complexity may not be sufficient to predict functionality of plants.

Conventional plant breeding has been successful for many years and has significantly contributed to providing food for the world population. Breeding is a dynamic process that needs to consider changing environments and modified human demands. Both natural and manmade environments influence genetic constellations and biodiversity. Thus, environment contributes itself to genome fluidity and diversity, which is the basic prerequisite that enables genetic improvement. In this changing world, stable plant growth performance is a high challenge that depends on the genetic and acquired capability of organisms for responding to divergent environments with homeostasis and physiologic plasticity. Heritability of stability parameters for yield is substantially lower than that of yield. The number of environments needed to achieve yield stability was estimated to be from 10 [1] to 200 [2]. Recently, Mühleisen *et al.* [3] reported that precise assessment of yield stability of individual genotypes requires phenotyping in at least 40 test environments. Thus, the challenge for safe food and feed production lies in the identification of more sophisticated parameters for deep phenotyping, which provides plants with the appropriate plasticity that finally can guarantee stable production in sustainable agriculture [4]. At the same time, 'parameter-specific' tool development for prediction and screening is becoming a crucial bottleneck for future breakthrough progress in breeding.

Identifying genotypes for stable growth performance—a novel strategic approach

Plant plasticity and stable growth performance are complex quantitative traits. Recent advances in understanding early steps in cell reprogramming, stress management and its morphophysiological consequences indicate a crucial role of mitochondria in cell and organism homeostasis and phenotype plasticity [5–15]. While photosynthesis is required to provide energy and carbon skeletons, mitochondria are central for organizing and directing energy and carbon-turnover to the metabolic routes leading to adaptive morphophysiology [6, 16, 17]. Although this view seems obvious, it has so far been almost neglected. Nevertheless, supporting data are available. Green microalgae, as unicellular photosynthetic organisms, can serve as an efficient experimental system for general studies on the relation between photosynthesis and adaptive biomass growth. In green microalgae, biomass growth was predicted by applying calorimetric measurements of the dependence of metabolism on nutritional status [18]. Under autotrophic conditions, biomass growth was clearly photosynthesis-driven and greater than under mixotrophic or heterotrophic conditions, in which the media supplies a portion or all of the carbohydrates. Although higher plants are autotrophs at the whole plant level, tissues and cells have diverse identities and contexts, where photosynthesis is neither the driving nor the limiting factor. Biomass shoot growth is considered relevant for yield in major crops, such as cereals [19] and tomato [20, 21]. In other plant species like carrot and beans, meristematic growth of other target tissues, for example, roots, is more important [22–24]. It is now well accepted that in general, mitochondria are central for environmental stress perception, transmission via cellular signaling and final organism responses. This was linked to their highly dynamic nature in shape, size, number and mobility ([25] and references therein). Also, chloroplast synthesis and activities within these organelles can be controlled by mitochondria [17, 26–28].

Temperature is a major determinant for plant selection, since environmental temperature is a key factor in determining plant growth performance. Thus, climate changes are expected to have significant influences on growth performance. The need to consider the temperature distribution of the target environment for plant growth (breeding for regions) is accepted in genetic selection. Maximum production will occur when the performance curve of a genotype matches (is congruent to) the temperature distribution of the environment during the growth season ([29], Hansen *et al.* in preparation). Thermal fitness of a genotype can be calculated as the overlapping area of the growth performance curve and the temperature distribution curve. The overlapping area of these curves can then be used to predict productivity under changing temperature conditions (Figure 1) ([29], Hansen *et al.* in preparation).

Calorespirometry measures heat and gas exchange from biological systems and provides a rapid means to obtain growth performance curves [30–33]. Calorespirometry was recently highlighted as an emerging technology in pre-breeding selection [14, 34, 35]. Nogales et al. [23] highlighted the importance of performing calorespirometric measurements in species-specific target tissues related to the final trait chosen for breeding [35]. For carrots, it was shown that genotype inbred lines can be discriminated for differential temperature-dependent growth performance. Genotypes that stopped growing at varying low temperatures were discriminated by measurements on the meristem of the tap roots [34] that are crucial for yield production in this species [22]. The CalOxy-FMAS method is being extended to measure the effects of growth-stimulating agents, including mycorrhizal fungi and other endophytes [25]. Analyses are also initiated that include major crops, such as cereals and tomato.

The cytochromic and alternative pathways in mitochondrial respiration provide the driving force for the chemical reactions that constitute growth. Plant tissue growth rate is equal to the rate of respiratory CO_2 production times an efficiency factor, $\varepsilon/(1\text{-}\varepsilon)$, where ε is the overall substrate carbon conversion efficiency. Calorespirometry is capable of near-simultaneous measurement of both the CO_2 rate and metabolic heat rate. [31, 32]. The substrate carbon conversion efficiency, ε, can be calculated from these two measurements [29, 30, 32]. Obtaining a complete temperature dependence curve for CO_2 rate, ε, and growth rate by calorespirometry on one tissue requires only about 1 day. No other method is capable of this. The respiratory heat rate is directly proportional to the rate of O_2 uptake [29] by the electron transport chain, and efficiency, ε, is hypothesized to be directly related to the ratio of activities of the alternative oxidase (AOX) and cytochrome oxidase (COX).

Fundamentally, together these two pathways provide the means to maintain optimal efficiency for ATP production [36] across a range of environmental conditions. Adaptive respiration seems to be linked to acclimation during the early phases of stress perception, but also plays a role during the later phases of stress translation into morphophysiological responses. Alternative respiration is known to be involved in cell homeostasis [5], and numerous papers are now available that report

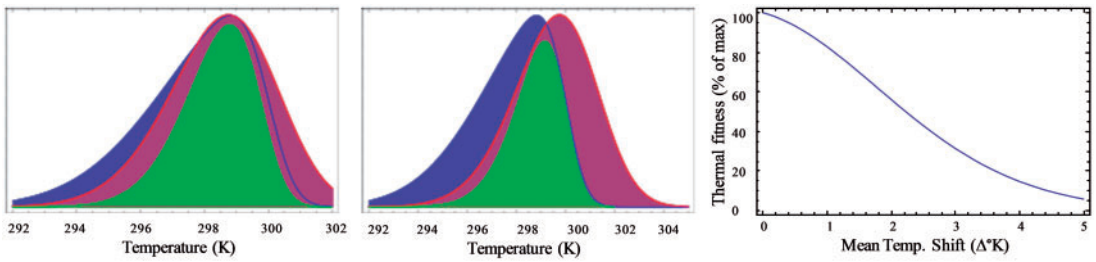

Figure 1. Left panel: Growth performance curve (at lowest T) and the temperature distribution (middle curve) are mostly congruent and the performance of the organism is near optimal. The product of the two curves is given by the enclosed area of overlap. The fitness of the organism is given by the integral of the overlap over the temperature range. Middle panel: The temperature distribution is shifted upward by 1K, the area of overlap and fitness is decreased. Right panel: Decrease in thermal fitness as a function of the change in mean temperature. (A colour version of this figure is available online at: http://bfg.oxfordjournals.org)

AOX involvement in growth and development as well as in the responses to diverse types of abiotic and biotic stresses (reviewed in [13]). Interestingly, alternative respiration seems also to be critical for the control of gas exchanges via stomata [37, 38], and thus is involved in controlling stomatal conductivity. This trait is used in current strategies for noninvasive, infrared thermography-based massive phenotyping and was shown to be adequate for genotype discrimination [39, 40].

This involvement of alternative respiration in homeostasis and stress management from a hierarchically upper position in metabolism was the reason why Arnholdt-Schmitt et al. [8] proposed *AOX* genes as a promising source for functional marker development [4, 41]. During the first hours of acclimation to new environmental conditions, Clifton *et al.* [42] showed that the alternative pathway was more variable than respiration through the cytochromic pathway. Shane *et al.* [16] showed that *AOX* was involved in exudation under P-depletion when secondary root clusters and growth of massive root hairs were induced. Early responsiveness of *AOX* genes could be confirmed by various *AOX* gene transcript accumulation studies connected to different severe cell reprogramming events with morphophysiological consequences under *in vitro* culture experimentation, such as growth induction from a quiescent tissue, during somatic embryogenesis and at rooting [9–11, 43]. The expression of *AOX* genes in carrot was upregulated before chilling exposure induced high transcript accumulation of the antifreezing gene for AFP, a specific pathway for plant protection against freezing (Campos et al. personal communication). However, so far we have little knowledge on how the two pathways and their key enzymes, COX and AOX, complement each other in diverse genotypes and under varying environmental stimuli and conditions. The significance of genetic differences for enzyme capacities versus enzyme engagement may be crucial for environmental changes that associate to acclimation and adaptation [44]. A superior genotype for yield stability needs not necessarily to show enzyme activities related to the actual growth but rather to the first steps of cell reprogramming (cell dedifferentiation/*de novo* differentiation), when cell destinies are determined (Campos et al. personal communication).

While *AOX* belongs to a small gene family that is encoded in the nucleus, COX consists of a large gene family that is transcribed mostly from mitochondrial DNA. Thus, co-regulation [43] and differential regulation [9] can contribute to overall enzyme activities and need to be considered when exploring molecular functionality at cell, tissue, organ and organism level.

Measuring oxygen isotope discrimination in the same tissue used for calorespirometry can provide additional information on genotype-specific AOX/COX ratio and on how this ratio can affect the genetic potential for growth rate and temperature response. This information can be valuable for distinguishing genotypes associated with stable growth performance. Additionally, oxygen isotope measurements will allow identification of the pathway in mitochondrial respiration (COX or AOX) [45 and references herein] that might be most important for robust growth performance and, therefore, at the same time most promising for identifying functional marker candidates.

Figure 2 highlights in its core circle, strategic experimental steps for predicting and validating genetic potential for stable plant growth performance. The outer circle in the figure indicates diverse superimposed levels of complexity and dynamics that contribute to genetic variability and can be explored by help of the proposed tool kit for breeding. In its core circle, experimental steps are highlighted to assist conventional and molecular breeding in predicting and validating genetic potential for stable plant growth performance. The experimental steps for genotype selection are as follows: (1) Selection of the tissue that is linked in a species-dependent manner to the desired final trait in breeding. (2) Rapid screening and selection of genotypes for temperature-dependent growth prediction by calorespirometry. (3) Validation of the results in the field. For further efforts toward FMAS, the following steps can be taken: (1) Determination of the AOX/COX ratio by oxygen isotope analysis. (2) Data mining from both tool components to associate AOX/COX ratio and predicted growth behavior in the selected genotypes. (3) Isolation of the associated *AOX* and *COX* gene sequences and their study at RT-qPCR and protein level during early and later stress responses on selected stress treatments to check linkage to general plant plasticity in target tissues for the final trait (e.g. growth regulation of the meristem ring in the tap root of carrots is directly linked to final yield [22, 34]). (4) Identification and characterization of the most promising target genes in the respiratory chain for functional marker development. (5) Applying calorespirometry on selected polymorphic genotypes to check for functional marker stability in view of growth prediction. For this, measurements need to be performed on plants during diverse developmental stages that grow under varying environments (temperature, nutrients, light regime, etc., considering also the severity of stresses). The outcome of applying CalOxy-FMAS will be a pool of preselected genotypes for final phenotyping in the field and a pool of candidate functional markers for the identification of unknown plant material from the same species. The data collected in a CalOxy-FMAS study allow exploring the effect of differences in the presence/absence of sequence motifs and genetic and epigenetic *AOX*- or *COX*-polymorphisms (SNPs, InDels, CNV, DNA methylation). Calorespirometry thus validates the functional relation of

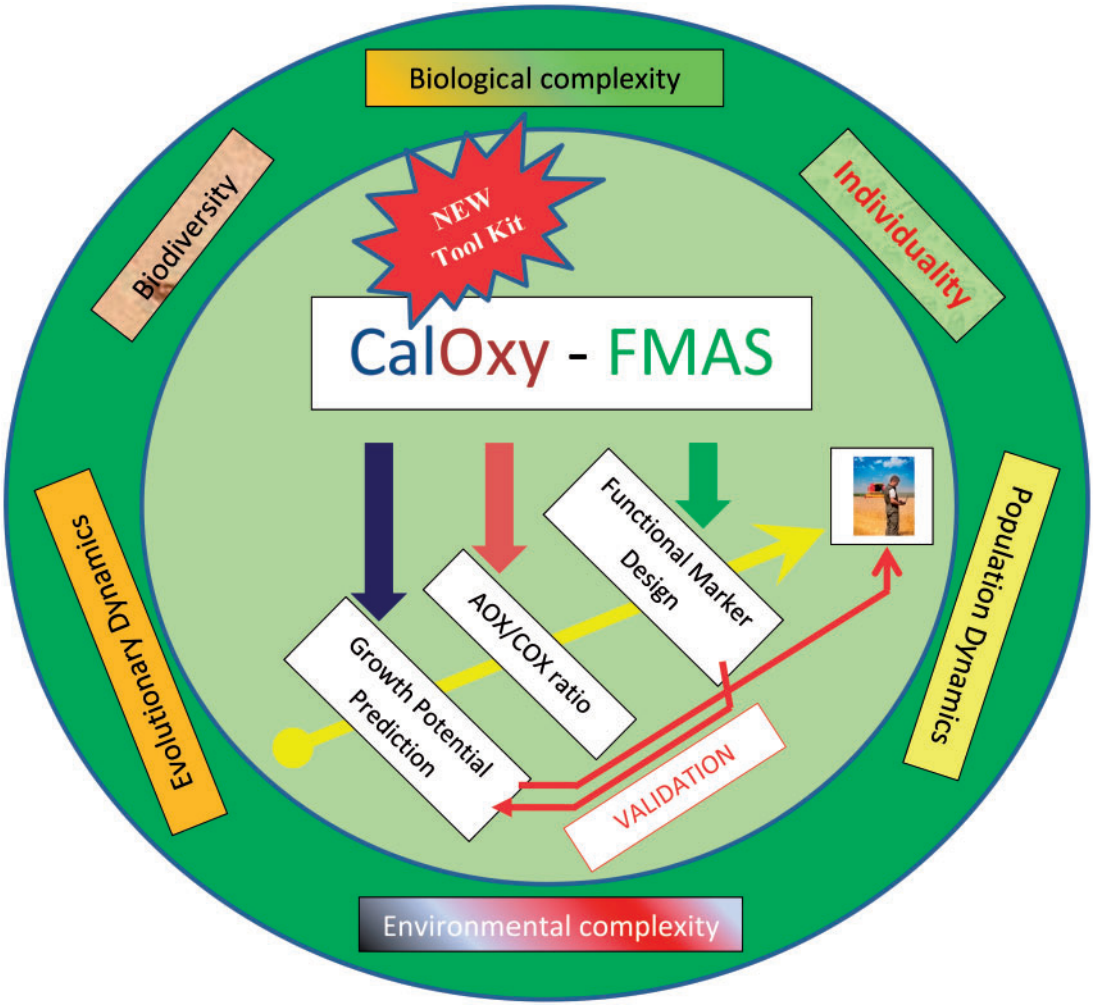

Figure 2. Parameter-specific pre-breeding tool 'CalOxy-FMAS' for predicting and validating genetic potential for stable plant growth performance. (A colour version of this figure is available online at: http://bfg.oxfordjournals.org)

DNA polymorphisms to the predicted growth performance before going to the field.

The special value of applying calorespirometry for validation of individual genotype and polymorphism functionality comes from the fact that it requires no knowledge of any details of the genetic or metabolic background of the measured genotypes. Calorespirometry measures the effect of complex individual constellations at organism as well as at cell, tissue and/or organ level, independent of the degree of biological complexity. This is also true when we consider plants as holobionts, i.e. the whole plant as a combination of the plant, which contributes the name for the whole, and its entire symbiont population. This combination constitutes the functional plant that adapts and 'is the organizational level at which natural selection acts. When challenged by environmental perturbations, a holobiont can use strategies unavailable to any one species alone' [46]. Holobionts show a discontinuous integration and interaction of hologenomes. The ubiquity of endophytes adds another important aspect of genetic complexity that can be further explored in breeding [25]. However, without a rapid development of appropriate new concepts and tools for intelligent screening, scientists will only get more and more lost in the unbearably large sources for data mining. The proposed 'CalOxy-FMAS' can expect to overcome this problem.

Key points

- Stability in plant growth performance demands extensive testing in diverse environments. A novel concept based on the role of respiration for homeostasis and adaptive morphophysiology is proposed to predict phenotypes with stable growth performance.
- Based on the novel concept the new tool 'CalOxy-FMAS', combining calorespirometry and oxygen isotope analysis for the validation of functional markers is proposed.
- The innovative approach is promising to identify functional markers in the background of diverse and complex genomes including hologenomes.
- The proposed concept and tool are appropriate to help closing the gap between functional genomics / hologenomics and plant improvement.

Acknowledgements

The Authors want to thank Prof. Gus Hart from the Physics Department of Birgham Young University for creating Figure 1.

Funding

The authors would like to acknowledge support by the Portuguese Foundation for Science and Technology (FCT) for three projects that enabled collaboration among the scientists: PTDC/AGR-GPL/111196/2009; EXCL/AGR-PRO/0038/2012; EXPL/AGR-FOR/1324/2013. The projects were supported by the program POPH—Operational Program for Human Potential and by FEDER Funds through the Operational Program for Competitiveness Factors—COMPETE. B.A.S. and A.N. appreciate support for research and Marie Curie Fellowships by the European Commission under the Grant-Agreement 251464 - AGRO-AMF-AOX.

References

1. Becker H.C. Zur Heritabilität statistischer Maßzahlen für die Ertragssicherheit [in German]. *Vortr Pflanzenzüchtg* 1987;**12**:134–44.
2. Piepho H.P. Methods for comparing the yield stability of cropping systems. *J Agron Crop Sci* 1998;**180**:193–213.
3. Mühleisen J, Piepho H-P, Maurer HP *et al*. Exploitation of yield stability in barley. *Theor Appl Gen* 2014;**127**:1949–62.
4. Cardoso HG, Arnholdt-Schmitt B. Functional marker development across species in selected traits. In: Lübberstedt T, Varshney RK (eds). *Diagnostics in Plant Breeding*, Vol. 21. Netherlands, Dordrecht: Springer, 2013, 467–15.
5. Hansen LD, Church JN, Matheson S, *et al*. Kinetics of plant growth and metabolism. *Thermochim Acta* 2002;**388**:415–25.
6. Arnholdt-Schmitt B. Stress-induced cell reprogramming. A role for global genome regulation? *Plant Physiol* 2004;**136**:2579–86.
7. Sieger, SM, Kristensen BK, Robson CA, *et al*. The role of alternative oxidase in modulating carbon use efficiency and growth during macronutrient stress in tobacco cells. *J Exp Bot* 2005;**56**:1499–515.
8. Arnholdt-Schmitt B, Costa JH, de Melo DF. AOX—a functional marker for efficient cell reprogramming under stress. *Trends Plant Sci* 2006;**11**:281–7.
9. Frederico AM, Campos MD, Cardoso HG, *et al*. Alternative oxidase involvement in Daucus carota somatic embryogenesis. *Physiol Plant* 2009;**137**:498–508.
10. Macedo ES, Cardoso HG, Hernandez A, *et al*. Physiologic responses and gene diversity indicate olive alternative oxidase as a potential source for markers involved in efficient adventitious root induction. *Physiol Plant* 2009;**137**:532–52.
11. Petrussa E, Bertolini A, Casolo V, *et al*. Mitochondrial bioenergetics linked to the manifestation of programmed cell death during somatic embryogenesis of *Abies alba*. *Planta* 2009;**231**:93–107.
12. Whelan SP, Zuckerbraun BS. Mitochondrial signaling: forwards, backwards, and in between. *Oxid Med Cell Longev* 2013;**2013**:351–613.
13. Vanlerberghe G.C. Alternative Oxidase: a mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants. *Int J Mol Sci* 2013;**14**:6805–47.
14. Arnholdt-Schmitt B. Introduction. Sub-chapter in Arnholdt-Schmitt B. From AOX diversity to functional marker development. In: Gupta KJ, Mur LAJ, Neelwarne B (eds). *Alternative Respiratory Pathways in Higher Plants*. Oxford: John Wiley & Sons Inc., 2015, in press.
15. Campos MD, Frederico AM, Nothnagel T, *et al*. Selection of suitable reference genes for reverse transcription quantitative real-time PCR studies on different experimental systems from carrot (*Daucus carota* L.). *Sci Hort* 2015;**10**:e0117569.
16. Shane MW, Cramer MD, Funayama-Noguchi S, *et al*. Developmental physiology of cluster-root carboxylate synthesis and exudation in *Harsh Hakea*. expression of phosphoenolpyruvate carboxylase and the alternative oxidase. *Plant Physiol* 2004;**135**:549–60.
17. Dahal K, Jia Wang, Greg D, *et al*. Mitochondrial alternative oxidase maintains respiration and preserves photosynthetic capacity during moderate drought in *Nicotiana tabacum*. *Plant Physiol* 2014;**166**:1560–74.
18. von Stockar U, Marison I, Janssen M, *et al*. Calorimetry and thermodynamic aspects of heterotrophic, mixotrophic, and phototrophic growth. *J Therm Anal Calorim* 2011;**104**:45–52.
19. Richards RA. Selectable traits to increase crop photosynthesis and yield of grain crops. *J Exper Bot* 2000;**51**:447–58.
20. Bolarín MC, Pérez-Alfocea F, Cano EA, *et al*. Growth, fruit yield, and ion concentration in tomato genotypes after pre- and post-emergence salt treatments. *J Am Soc Hort Sci* 1993;**118**:655–60.
21. Van der Ploeg A, Heuvelink E. Influence of sub-optimal temperature on tomato growth and yield: a review. *J Hort Sci Biotech* 2005;**80**:652–9.
22. Arnholdt-Schmitt B. On the physiology of yield production in carrots- Implications for breeding towards nutrient efficiency. *Gartenbauwissenschaft* 1999;**64**:26–32
23. Nogales A, Muñoz-Sanhueza L, Hansen LD, *et al*. Calorespirometry as a tool for studying temperature response in carrot (*Daucus carota* L.). *Eng Life Sci* 2013;**13**:541–8.
24. Araújo AP, Teixeira MG. Relationships between grain yield and accumulation of biomass, nitrogen and phosphorous in common bean cultivars. *R Bras Ci Solo* 2008;**32**:1977–86.
25. Arnholdt-Schmitt B, Valadas V, Döring M. Functional marker development is challenged by the ubiquity of endophytes- a practical perspective. *Brief Funct Genom* 2016;**15**:16–21.
26. Hoefnagel MHN, Atkin OK, Wiskich JT. Interdependence between chloroplasts and mitochondria in the light and the dark. *Biochim Biophys Acta* 1998;**1366**:235–55.
27. Raghavendra AS, Padmasree K. Beneficial interactions of mitochondrial metabolism with photosynthetic carbon assimilation. *Trends Plant Sci* 2003;**8**:546–53.
28. Yoshida K, Watanabe CK, Terashima I, *et al*. Physiological impact of mitochondrial alternative oxidase on photosynthesis and growth in *Arabidopsis thaliana*. *Plant Cell Environ* 2011;**34**:1890–9.
29. Hansen LD, Thomas NR, Arnholdt-Schmitt B. Temperature responses of substrate carbon conversion efficiencies and growth rates of plant tissues. *Physiol Plant* 2009;**137**:446–58.
30. Criddle RS, Smith BN, Hansen LD. A respiration based description of plant growth rate responses to temperature. *Planta* 1997;**201**:441–5.
31. Criddle RS, Hansen LD, Smith BN, *et al*. A thermodynamic law of adaptation of plants to environmental temperatures. *Pure Appl Chem* 2005;**77**:1425–44.
32. Hansen LD, Criddle RS, Smith BN. Calorespirometry in plant biology. In: Lambers H, Ribas-Carbo M (*eds*). *Plant Respiration: from Cell to Ecosystem, Advances in Photosynthesis and Respiration*. Dordrecht: Springer, 2005, 17–30.
33. Wadso L, Hansen LD. Calorespirometry of terrestrial organisms and ecosystems. *Methods* 2015;**76**:11–9.
34. Nogales A, Muñoz-Sanhueza L, Hansen LD, *et al*. Phenotyping carrot (Daucus carota L.) for yield-determining temperature response by calorespirometry. *Planta* 2014, **241**:525–38. doi:10.1007/s00425-014-2195-y.
35. Nogales A, Noceda C, Ragonezi C, *et al*. Discovering the relevance of AOX gene diversity: Functional marker development from AOX genes requires deep phenotyping and individualized diagnosis. In: Gupta KJ, Mur LAJ, Neelwarne B (*eds*).

Alternative Respiratory Pathways in Higher Plants. Oxford: John Wiley & Sons, Inc., 2015, in press.
36. Stucki JW. Optimization of mitochondrial energy conversions. In: Nicolis G (*ed*.). *Advance in Chemical Physics*, Vol. LV. John Wiley & Sons Inc, 1984.
37. Gupta KJ, Mur LAJ, Ratcliffe RG. Guarding the guard cells? *New Phytol* 2014;**203**:349–51.
38. Cvetkovska M, Dahal K, Alber NA, *et al*. Knockdown of mitochondrial alternative oxidase induces the 'stress state' of signaling molecule pools in *Nicotiana tabacum*, with implications for stomatal function. *New Phytol* 2014;**203**: 449–61.
39. Prashar A, Yildiz J, McNicol JW, *et al*. Infra-red thermography for high throughput field phenotyping in *Solanum tuberosum*. *PLoS One* 2013;**8**:e65816.
40. Rogiers SY, Greer DH, Hatfield JM, *et al*. Stomatal response of an anisohydric grapevine cultivar to evaporative demand, available soil moisture and abscisic acid. *Tree Physiol* 2012;**32**: 249–61.
41. Polidoros AN, Mylona PV, Arnholdt-Schmitt B. *AOX* gene structure, transcript variation and expression in plants. *Physiol Plant* 2009;**137**:342–53.
42. Clifton R, Lister R, Parker KL, *et al*. Stress-induced co-expression of alternative respiratory chain components in Arabidopsis thaliana. *Plant Mol Biol* 2005;**58**:193–212.
43. Campos MD, Cardoso HG, Linke B, *et al*. Differential expression and co-regulation of carrot *AOX* genes (*Daucus carota*). *Physiol Plant* 2009;**137**:578–91.
44. Macfarlane C, Hansen LD, Florez-Sarasa I, *et al*. Plant mitochondria electron partitioning is independent of short-term temperature changes. *Plant Cell Environ* 2009; **32**:585–91.
45. Kornfeld A, Horton TW, Yakir D, *et al*. A field-compatible method for measuring alternative respiratory pathway activities in vivo using stable O_2 isotopes. *Plant Cell Environ* 2012; **35**:1518–32.
46. Gordon J, Knowlton N, Relman DA, *et al*. 2015. Superorganisms and Holobionts. Looking for a term for the functional entity formed by a macrobe and its associated symbiotic microbes and viruses? The term is "holobiont". Microbe Magazine. Ame Soc Microbiol. http://www.microbemagazine.org/index.php?option=com_content&view=article&id=6300:superorganisms-and-holobionts&catid=1205&Itemid=1464.